Plant Pathology in the 21st Century

Peter Scott · Richard Strange
Lise Korsten · Maria Lodovica Gullino
Editors

Plant Diseases and Food Security in the 21st Century

Springer

Plant Pathology in the 21st Century

Volume 10

Series Editor

Maria Lodovica Gullino, Università degli Studi di Torino, AGROINNOVA, Grugliasco, Torino, Italy

The aim of this Series is to highlight the latest international findings and advances in plant pathology and plant disease management, and plant pathology topic specialist, Congress and Workshop organisers, coordinators of broad International projects are invited to consult with the Series Editor regarding their topic's potential inclusion in the series.

Under an agreement with Springer, a Book Series based on the invited lectures at the 9th International Congress of Plant Pathology ICPP2008, *Plant Pathology in the 21* st *Century* was initiated and four books covering key themes were published. Following on the procedure for the 2008 Congress, three additional volumes in the series were published on themes which were key topics at ICPP2013, held in Beijing, China, while two new books are almost ready from ICPP 2018 held in Boston (SA). Moreover, more books were published in between Congresses, covering up-to-date topics in plant pathology. In light of the initial seven volumes' success, the ISPP has now concluded an agreement with Springer to broaden the scope of the Series and publish additional volumes.

The goal of the International Society for Plant Pathology (ISPP; www.isppweb.org) is to promote the global advancement of plant pathology and the dissemination of essential information on plant diseases and plant health management. This book Series looks of particular interest due to the upcoming International Year of Plant Health (2020).

More information about this series at http://www.springer.com/series/8169

Peter Scott • Richard Strange
Lise Korsten • Maria Lodovica Gullino
Editors

Plant Diseases and Food Security in the 21st Century

Springer

Editors
Peter Scott
International Society for Plant Pathology
Oxford, UK

Lise Korsten
Department of Plant Sciences
University of Pretoria
Pretoria, South Africa

Richard Strange
Department of Genetics, Evolution and Enivironment
University College London
London, UK

Maria Lodovica Gullino
Università di Torino
Centro Agroinnova
Grugliasco, Italy

ISSN 2512-160X ISSN 2512-1626 (electronic)
Plant Pathology in the 21st Century
ISBN 978-3-030-57898-5 ISBN 978-3-030-57899-2 (eBook)
https://doi.org/10.1007/978-3-030-57899-2

© Springer Nature Switzerland AG 2021
Chapters 5 and 11 are licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). For further details see licence information in the chapters.
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Cover Illustration: Orange fleshed sweetpotato variety 'Kakamega', showing severe symptoms of SPVD, next to a symptomless plant. Photo: courtesy of Segundo Fuentes.

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

Plant pathologists are confronting unprecedented global challenges as we move through the 21st century. These challenges include huge population increases, expanded global trade, unpredictable changes in the climate, and degradation of the lands and water needed for agriculture. In many countries and regions, we face the additional challenges of changing consumer food preferences, which include increasing demands for safe and nutritious foods as well as fresh, local, and organically produced foods. Overall, this means that pathologists cannot focus only on the staple crops for food security. Providing nutritious diets while considering environmental health and stable agricultural systems will require improved, innovative, and broadly applicable approaches and strategies to reduce the overall impacts of plant disease.

An overarching problem for pathologists is that solid, validated data on how the expected future challenges will impact a given disease on a particular crop are available for only a very few interactions. We don't know how environmental stresses (drought, high temperatures, salinity, etc.) will affect the plants and/or the pathogenic or beneficial microbes in the system. That is, we don't yet have accurate information to predict how the phytobiome will respond. For example, for some host/pathogen systems, increasing temperatures lead to more disease, whereas in others, the opposite is true. For some host/pathogen interactions, increasing temperatures render some sources of disease resistance ineffective, resulting in disease, whereas other resistance sources are more effective at high temperatures, and the plants are more resistant to disease. We are just beginning to understand how various microbial components of microbiomes contribute to improved soil and plant health, and which combinations can help to remediate plant disease. While many innovations and technological improvements have been made that will contribute to solving these complex problems, to understand the outcomes for crops critical to a secure and sustainable future, we will need significant collaborative efforts and investments.

The book series Plant Pathology in the 21st Century, coordinated by the International Society for Plant Pathology and the Series Editor Maria Lodovica Gullino, is an important *call to arms* for plant pathologists, identifying the

conceptual, tangible, and technical challenges we face in this century. The series is intended to motivate consideration of the challenges we face in this century. It is entirely appropriate that this edition in the series Plant Diseases and Food Security in the 21st Century be published now, given the proclamation of 2020 as the International Year of Plant Health.

This book in the series reminds us that all of us are impacted by crop diseases, existing or emerging, from our favorite breakfast foods to the staple crops considered essential to food security. Contributing authors use diverse examples to address innovative ways to detect, monitor, and manage plant diseases, and how to model and predict the impacts of diseases on our food system. In recognition of the broader scope of food security, the book contains chapters that address the safety of foods, from pesticide contamination to transmission of human pathogens. All of these factors, including the tools, methods, and innovative strategies, are important, but for successful disease management in the future, we will need robust extension and outreach systems armed with relevant information such that they can transmit actionable guidance to the crop managers and farmers.

As old and new diseases emerge and re-emerge, and changing environmental conditions compound the problems, the global conversations highlighted in this book and others in the series are important steps, but only the first steps. How we, as individuals or as communities of plant pathologists, respond to these needs for action will be important in ensuring food safety and security through the twenty-first century.

President, International Society for Plant Pathology Jan E. Leach
Agricultural Biology, Colorado State University,
Fort Collins, CO, USA

Preface

This book is an initiative of the International Society for Plant Pathology (ISPP). It is the 10th in ISPP's book series Plant Pathology in the 21st Century. As part of each of the last five International Congresses of Plant Pathology (1998, 2003, 2008, 2013 and 2018), ISPP's Commission on Global Food Security has coordinated sessions focusing on plant disease as a factor limiting global food security. This book is based on those sessions at the 11th International Congress of Plant Pathology, held in Boston, USA, in 2018, sponsored by ISPP and organized by the American Phytopathological Society.

The editors are grateful for the opportunity to bring together, in this volume, chapters based on a wide variety of presentations at the Congress – bearing on the persistent need for *action* to address the challenges to global food security posed by plant pathogens.

Special thanks are due to Jean Ristaino for coordination of these Congress presentations. We are grateful to the Canadian Phytopathological Society for permission and encouragement to reproduce the Glenn Anderson Lecture as Chap. 3. We also thank the authors of each chapter for their cooperation in making this book.

There are five parts to the book. The first relates to discussions of an expert panel, open to the public. The others relate to four of the formal sessions of the Congress that focused on plant disease and food security.

The book opens with potato blight and the crisis of the 1845 Irish potato famine. E. C. Large's 1940 book *The Advance of the Fungi* opens here too, with a vivid account of Ireland's blighted potato crops and of the continuing struggle to manage the disease. The present book addresses this struggle in the context of food security in the twenty-first century. We have the benefit of greatly enhanced understanding of plant disease mechanisms. Nevertheless, we are inclined to share the realism of Large's 1940 vision of a continuing struggle. The final lines of his book are:

> *Those who believed in genes postulated the existence of an eternal quality, R, which they could take from wild plants, build into the genetical constitution of cultivated ones, and so make them disease-resistant for ever. Those who thought, not in terms of mathematical abstractions, but of the green flux of ever-changing nature, saw little hope of such permanency, and no end to man's labours in defending the crops upon which he depended for life.*

At present, we have a constant reminder of how insidious disease can be, in the form of Covid-19, a viral pathogen of humans, and how failure to acknowledge it promptly turned what could have been a localized epidemic into a pandemic. With plant diseases that affect our crop plants and therefore food security, it is not so much a failure to acknowledge them but the difficulty of identifying their causal agents and finding appropriate techniques to control them. In the first place, there are many species of crop plants that are of importance sociologically and economically, and each has a suite of pathogens. Then, compared with human pathologists, who mainly have to consider only one host organism, humans, there are multiplicities of hosts and pathogens and relatively few plant pathologists to cope with them! However, cope we must if we are to attain the second of the United Nations Sustainable Development Goals – zero hunger by 2030. Already we have over 820 million people who are hungry and over 2 billion who are suffering from hidden hunger – a lack of one or more vitamins or trace elements – and these numbers, far from diminishing, are increasing.

A first task and a fundamental one for controlling plant disease is accurate identification of the causal agent. Not surprisingly, diagnostics feature to varying degrees of prominence in nearly half the chapters in the book. Once the identity of a causal agent of a disease has been established, it is necessary to find a rapid and reliable test, preferably one that can be used in the field, to monitor its occurrence and establish its epidemiology. Speedy action may allow confinement of the disease to a limited area and possibly even its eradication. Second, some assessment of the social and economic costs of the disease should be obtained in order to prioritize the use of usually scarce resources in management and control. Third, if the disease is causing serious social and economic costs, management and control measures must be established to limit these.

Before taking these three points in turn, we would like to draw readers' attention to Chap. 3, which is essentially a biography of Glenn Anderson, a Canadian, who made tremendous contributions to wheat breeding, pathology and the training of young scientists. Norman Borlaug referred to him as his 'green-fingered agricultural scientist' and his General 'Eisenhower' on the frontline in Asia who launched the 'Green Revolution'. He headed the Coordinated International Wheat Program for the Rockefeller Foundation, the Indian Council of Agricultural Research and later, after its creation, the International Maize and Wheat Improvement Center (CIMMYT) in India, 1964–1970. In 1971, he moved to Mexico, becoming Associate Director and, on Norman Borlaug's retirement, Director of CIMMYT. Being a 'doer' he realized that he 'needed boots on the ground' in the wheat-growing areas of less developed countries. As a result, 6-month-long field-orientated training courses for young international staff were initiated in the late 1960s and continued into the 1970s. These were the germ of regional programs in the Andean region and southern cone of South America, North Africa, Middle East, East Africa and West Africa. Sadly, Glenn's life was cut short by leukaemia – but what a legacy he has left in so many parts of the world!

Turning now to diagnostics, a number of novel techniques are described. Chapter 1 starts with an account of potato blight and its profound effects wrought on Ireland

in the 1840s – starvation and mass emigration. Nobody knew at the time what the cause of 'the potato murrain' was, but this was not surprising considering that it predated Pasteur's germ theory of disease. In this chapter, Jean Ristaino and colleagues, using specimens from the original outbreak, amplified three mitochondrial genes and, using Single Nucleotide Polymorphisms (SNPs), were able to show that the 1a mtDNA haplotype was responsible and not the 1b as was originally thought. This work demonstrates the power of molecular techniques in determining lineages of pathogens.

In Chap. 9, Sara Franco Ortega and colleagues call attention to the use of novel serological and molecular diagnostic techniques, exemplified by three important fungal pathogens of rice. They suggest that the LAMP (loop-mediated isothermal AMPlification) assay for on-site detection of pathogens, combined with alkaline extraction of DNA, has great potential for assessing inoculum, including inoculum that is airborne.

Jonathan Shao and colleagues (Chap. 10) describe the use of immune tissue prints and machine learning to detect the causal organism of citrus greening disease, also known as huanglongbing. The organism is an unculturable member of the alpha-proteobacteria '*Ca*. Liberibacter asiaticus'. Cut ends of stems or petioles are pressed onto nitrocellulose paper and a fragment of an outer membrane of the pathogen is detected on the paper by a rabbit polyclonal antibody. A goat anti-rabbit antibody conjugated with alkaline phosphatase and substrate for the enzyme then detects the rabbit antibody. Positives show as dark dots in the phloem. Details of the means by which the tissue prints could be scored using machine learning are given. This allows mass screening of plant material, a necessity for a pathogen that has non-uniform distribution in infected trees.

Three chapters are concerned with the social and economic cost of plant disease. Kreuze et al. (Chap. 5) point out that when sweet potato was introduced from the Americas to Africa over 500 years ago, the varieties were white- or yellow-fleshed, containing no or little beta-carotene, respectively. Beta-carotene is a precursor of vitamin A, which is converted by human metabolism to the vitamin. Sadly but avoidably in sub-Saharan Africa (SSA), 48% of children are estimated to be vitamin A deficient (VAD) – a high social cost as, among other negative effects, VAD is the leading cause of preventable blindness in young children. Efforts are therefore being made to introduce orange-fleshed sweet potato (OFSP), which has high concentrations of beta-carotene, into SSA. Unfortunately, viral diseases have frustrated these attempts. It is a cruel irony that endeavours to counteract the high incidence of VAD in SSA by introducing OFSP are being frustrated by viral infection – more than 30 different viruses are known to infect sweet potato, some in combination completely destroying the crop.

In Chap. 6 Castilla and colleagues review the impact of rice diseases in tropical Asia. They describe a yield- loss model, RICEPEST, originally designed to simulate rice crop growth and development along with injury mechanisms due to pathogens, insects and weeds but sufficiently flexible to be used for other crops. In experiments for Asia overall, mean yield losses owing to diseases, animal pests and weeds were 37.2%.

Epidemic modelling at the landscape level is described with mathematical detail by Fabre and co-authors in Chap. 4. They point out that increased global travel and trade as well as changing climatic patterns have led to the emergence of new and more frequent plant disease epidemics. Mathematical modelling can predict how and where these new arrivals will spread and thus inform and optimize their control. Coding is provided to allow readers to run models for themselves.

In Chap. 7, Petronaitis and colleagues describe the importance of Fusarium diseases of wheat. These are Fusarium headblight and Fusarium crown rot, which are caused by various species of the genus, some of which are also associated with ear, stalk and root rot of maize. The authors provide comprehensive tables showing heavy losses in most wheat-growing areas of the world. Another factor of importance is the production of mycotoxins by these fungi, which killed nearly 100,000 people in Russia in the 1940s (Pitt and Miller 2017). Mycotoxin contamination of food is insidious because dangerous – if not lethal – concentrations may be present in crops such as cereals and legumes – and remain there undetected. One poignant case is the presence of aflatoxin in infant formulations in Ghana. Here, about 40% of deaths of children under 5 years are attributed to malnutrition. In order to counteract this, ready-to-use cereal-legume blends have become available on the market but some contain five times the acceptable limit of 20 ppb aflatoxin. Thus, a policy to improve infant nutrition may have worsened their health (Opoku et al. 2018).

Turning now to disease management, Claire Beverley and Manju Thakur (Chapter 11) point out that 500 million smallholder farmers provide over 80% of the food for a large part of the developing world but they face considerable losses to plant disease. Even if these losses were reduced by as little as 1%, millions more could be fed. They describe a valuable ICT (Information and Communication Technology) tool, which helps farmers to manage pests and diseases of their crops and obtain local news about those that are currently causing concern.

Of course, not allowing pathogens into an area where they are absent is the ultimate disease control method, but with increased travel of people (although currently limited owing to Covid-19) and plants traded widely as commodities, this is becoming increasingly difficult. Mike Jeger and associates (Chap. 8) describe four stages in assessing risks to plant health by the introduction of foreign pests or pathogens: Introduction, Establishment, Spread and Consequences. The European Union's Scientific Panel on Plant Health originally used qualitative methods to assess these four stages but, as explored in the chapter, now provides guidance for their quantitative assessment.

Genetic resistance is the preferred method of control in many instances but it is not always feasible. For example, there are few sources of resistance in *Citrus* to huanglongbing, and breeding a new cultivar by conventional methods may take 20 years (Chap. 1). Genetic modification (GM) could provide a solution but, as is described below, faces stringent regulatory barriers to adoption. In this case, control of the psyllid vector, *Diaphorina citri,* offers an opportunity but involves numerous applications of insecticide, regular scouting and rogueing of infective trees.

Ploetz (Chap. 2) points out that there are convincing arguments for using GM (although it is a technique banned by several countries of the European Union) to

produce genotypes of premium bananas such as Cavendish with resistance to Fusarium wilt. For example, Grand Nain, a Cavendish cultivar, transformed with a nematode-derived gene, Ced9, remained resistant even to the much-feared TR4 strain of the causal organism during a 3-year trial. However, a line transformed with the RGA2 gene from another *Musa* species, *Musa acuminata* ssp. *malaccensis,* also showed resistance in the trial. Homologs of the RGA2 gene are present in wildtype Grand Nain but were expressed at levels 10-fold lower than in the most resistant transgenic lines. Dale et al. (2017) suggested that the expression of RGA2 homologs 'might "be elevated through gene editing, to provide non-transgenic resistance", which would therefore avoid anti-GM scrutiny'. No doubt the authors were disappointed to learn that, in 2018, the European Court of Justice characterized gene editing as a form of GM, showing that the Court is incapable of distinguishing a technique from its product. Of course, any new variety of crop plant should be tested rigorously for unwelcome properties, but to deny millions of the world's still increasing population the benefits of important crop varieties simply because of the way they are produced seems crass.

The last two chapters of the book are concerned with food safety. Nicola Holden (Chap. 13) draws attention to the fact that edible plants can be important vehicles for the transmission of human pathogens and that there are molecular parallels between human pathogens and plant pathogens in the triggering of defence responses in their hosts. She suggests that such parallels could be exploited in shared dialogue and research.

Carmen Tiu (Chap. 12) points out the necessary role that pesticides play in integrated pest management of our crops, and therefore in food security, but emphasizes the importance of limiting the unwanted traces they may leave in food. Unacceptable levels of pesticide residues are defined by Maximum Residue Limits (MRLs). The effectiveness of MRLs depends on widespread acceptance, for which simpler protocols are urgently needed: an outline scheme is proposed.

Readers of this book will find it contains much food for thought, which – it is hoped – will be transformed into food for people and achievement of SDG2 – zero hunger by 2030. For this, to use Glenn Anderson's phrase, 'boots on the ground' will be needed.

Oxford, UK — Peter R. Scott

London, UK — Richard Strange

Pretoria, Republic of South Africa — Lise Korsten

Torino, Italy — Maria Lodovica Gullino

References

Dale, J., James, A., Paul, J. Y., Khanna, H., Smith, M. ,Peraza-Echeverria, S., Garcia-Bastidas, F., Kema, G., Waterhouse, P., Mengersen, K., & Harding, R. (2017). Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nature Communications, 4*, 1–8

Opoku, N., Achaglinkame, M. A., & Amagloh, F. K. (2018) Aflatoxin content in cereal-legume blends on the Ghanaian market far exceeds the permissible limit. *Food Security, 10*, 1539–1545.

Pitt J. I., & Miller J. D. (2017). A concise history of mycotoxin research. *Journal of Agriculture and Food Chemistry, 65*, 7021–7033

Contents

Part I Crop Diseases Threaten Global Food Security and Your Breakfast

1 Potatoes, Citrus and Coffee Under Threat 3
Jean Beagle Ristaino

2 Gone Bananas? Current and Future Impact of Fusarium Wilt on Production 21
Randy C. Ploetz

Part II Emerging Plant Diseases and Global Food Security

3 Plant Diseases, Global Food Security and the Role of R. Glenn Anderson 35
S. Rajaram and H. J. Dubin

4 Optimising Reactive Disease Management Using Spatially Explicit Models at the Landscape Scale 47
Frédéric Fabre, Jérôme Coville, and Nik J. Cunniffe

5 Challenge of Virus Disease Threats to Ensuring Sustained Uptake of Vitamin-A-Rich Sweetpotato in Africa 73
Jan Kreuze, Wilmer J. Cuellar, and Jan W. Low

Part III Global Impacts of Plant Disease Epidemics

6 The Impact of Rice Diseases in Tropical Asia 97
N. P. Castilla, J. B. Macasero, J. E. Villa, A. H. Sparks, L. Willocquet, and S. Savary

7 Importance of *Fusarium* spp. in Wheat to Food Security: A Global Perspective 127
Toni Petronaitis, Steven Simpfendorfer, and Daniel Hüberli

8 Quantitative Assessment of Consequences of Quarantine Plant Pathogen Introductions: From Crop Losses to Environmental Impact 161
Michael Jeger, Giuseppe Stancanelli, Gianni Gilioli, Gregor Urek, Ariena van Bruggen, Jean-Claude Grégoire, Vittorio Rossi, Wopke van der Werf, Alan MacLeod, Gritta Schader, Sybren Vos, Svetla Kozelska, Marco Pautasso, Ciro Gardi, and Olaf-Mosbach-Schulz

Part IV Innovative Techniques for Monitoring Emerging Diseases

9 Diagnosis and Assessment of Some Fungal Pathogens of Rice: Novel Methods Bring New Opportunities 195
Sara Franco Ortega, Davide Spadaro, and Maria Lodovica Gullino

10 Automated Detection of '*Ca*. Liberibacter asiaticus' Infection in Citrus Using Immune Tissue Prints and Machine Learning 215
Jonathan Shao, Fang Ding, Shimin Fu, and John Hartung

11 Plantwise: A Knowledge and Intelligence Tool for Food Security through Crop Protection 231
Claire Beverley and Manju Thakur

Part V Plant Diseases and Food Safety

12 Pesticide Residues in Food: A Never-Ending Challenge 251
Carmen Tiu

13 How Can Plant Pathology Help in the Control of Human Pathogens Associated with Edible Crop Plants? 259
Nicola Holden

Index 277

Part I
Crop Diseases Threaten Global Food Security and Your Breakfast

Chapter 1
Potatoes, Citrus and Coffee Under Threat

Jean Beagle Ristaino

Abstract Four familiar breakfast foods, coffee, oranges, bananas and potatoes provide current examples of the challenge to food security posed by plant diseases. Potato late blight, a disease with a historical link to famine and a continued threat to a staple crop; huanglongbing, a devastating disease that threatens the citrus industry; coffee leaf rust, a disease that compromises a commodity and the livelihoods of those who service it; and Fusarium wilt of bananas, a globally damaging disease with a virulent race that is currently spreading. What if these morning staples were to become scarce or unavailable? This chapter reflects the discussions of an expert panel at a public meeting, held as part of the 2018 international Congress of Plant Pathology at Harvard Museum of Science and Culture. The focus of the panel that included specialists in plant pathology and in food security, is on emerging diseases, covering topics that include the evolution of plant pathogens, tracking how they spread around the globe, and strategies to combat plant diseases that are threatening global food security.

Introduction

Coffee, oranges, bananas and potatoes are among the most widely consumed breakfast foods. What if these morning staples were to become scarce or unavailable? In this opening section of the book we focus on familiar foods to provide current examples of the challenge to food security posed by plant diseases.

At the 2018 International Congress of Plant Pathology, in Boston MA, an expert panel was convened in a packed evening session open to the public at Harvard Museum of Science and Culture to consider the impact of four such diseases that currently present severe threats to food security (Fig. 1.1).

J. B. Ristaino (✉)
Department of Entomology and Plant Pathology, Emerging Plant Disease and Global Food Security Cluster, North Carolina State University, Raleigh, NC, USA
e-mail: jbr@ncsu.edu

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_1

Fig. 1.1 The panel (left to right): Megan M. Dewdney, University of Florida; Randy C. Ploetz, University of Florida; Angela Records, USAID, Washington DC; Gary D. Foster, University of Bristol; and Jean B. Ristaino, NC State University

- **Potato blight**[1]
  A disease with a historical link to famine and ongoing potential to threaten a staple crop
- **Huanglongbing of citrus - Megan M. Dewdney**[2]
  Probably the most devastating citrus disease – it threatens an industry
- **Coffee leaf rust - Angela Records and Jacques Avelino**[3]
  A disease that compromises a commodity and the livelihoods of those who service it
- **Fusarium wilt of banana - Randy Ploetz**
  A globally damaging disease with a virulent race that is currently spreading.

To capture a flavour of this public event the book opens with an account of a crime scene, with evidence left by the Irish potato famine of what came to be recognized as an epidemic of a plant disease and its culprit – potato blight.

This is followed by brief overviews of two currently critical diseases of citrus and coffee – huanglongbing and leaf rust.

The next chapter tells the unfinished story of the advance of tropical race 4 of Fusarium wilt of banana.

[1] The name "potato blight", or just "blight", is used informally in this chapter for the disease caused by *Phytophthora infestans*, responsible for the Irish potato famine. The disease is more formally known as "potato late blight", to distinguish it from "potato early blight", a different disease caused by *Alternaria solani.*

[2] Based on the panel presentation by Megan M. Dewdney, University of Florida, Lake Alfred, FL, USA.

[3] Based on the panel presentation by Angela Records, USAID, Washington, DC, USA and Jacques Avelino, CIRAD-CATIE-PROMECAFE, Coronado, Costa Rica.

Each of these diseases poses immediate threats not only to a crop but also to the livelihoods of hundreds of thousands of people involved in their cultivation and in food production. The focus of our panel – specialists in plant pathology and in food security – was on emerging diseases, covering topics that include the evolution of plant pathogens, tracking how they spread around the globe, and strategies to combat plant diseases that are threatening global food security.

Crime Scene Investigations: CSI Dublin – Tracking a Potato Killer

The Irish potato famine of the 1840s was the result of potato late blight, a serious disease of potatoes caused by the plant pathogen *Phytophthora infestans* (Fig. 1.2). The Irish famine was not only an issue of food security; it also threatened the national security of Ireland. The Irish working poor were highly dependent on potatoes as their staple food and when the crop failed, many people starved. The dependence on potatoes was remarkable: an adult male would consume 9–11 pounds of potatoes per day, with little diversity in diet. Though other crops such as oats and wheat were grown in Ireland in the nineteenth century they were largely exported to England. Only after the magnitude of the famine emerged was food aid increased to Ireland (Bourke 1993). The potato blight threatened both food security and Ireland's national security.

Fig. 1.2 Symptoms of blight caused by *Phytophthora infestans* on potato

When there was not enough food to go round and no surplus that could be sold to pay the rent, families were evicted from their homes. The Irish crime scene expanded as the potato crop failed. Many people moved into workhouses where human diseases were rife. A million Irish lives were lost during the potato famine through hunger and disease, especially among the poorest people. An additional two million left the country in mass emigration, often in unsuitable ships, with further loss of life. Conditions on these "coffin ships" and subsequent quarantine of the immigrants in places such as Grosse Ile in Quebec resulted in the spread of human diseases and thousands more perished *en route*. At a mass burial site in Grosse Ile, over 5000 Irish immigrants were buried after they died from disease.

The 2018 International Congress of Plant Pathology was held in Boston MA, where many of the surviving Irish emigrants settled. A monument to the Irish famine in Harvard Square declares "Never again should a people starve in a world of plenty" (Fig. 1.3). Regrettably, food security remains a huge problem in the twenty-first century, globally and in areas of the USA. Plant diseases continue to make a major contribution to the challenge of food security.

Fig. 1.3 Statue commemorating the Irish immigrants in Harvard Square, Boston

Naming the Culprit – Phytophthora, the "Plant Destroyer"

When the potato famine struck there was little understanding that pathogens cause disease. Who was the suspect in in this crime scene? The disease first occurred in 1843 in the USA. In 1845 it was reported in Europe and the British Isles. The 1843 Commissioner of Patents report (Ellsworth 1843) said, "The potato crop has been attacked. The cause is generally attributed to the peculiarity of the weather". Others claimed "It was certain that a fungus appeared in the leaves and tubers but it was uncertain how far the fungus was the cause or consequence of the disease" (Johnson 1845). Theories on the cause included bad air, the wrath of God, racial prejudice against the Irish, or a minute fungus (Fig. 1.4) (Ristaino et al. 2020). The British mycologist Miles Joseph Berkeley observed a characteristic microorganism on diseased potatoes, but was this the cause of the disease or an incidental result of some other cause (Berkeley 1846)? He published a detailed description of the disease, drew pictures of the pathogen and collected plant samples which remain in Kew Gardens Mycological Herbaria (Ristaino 1998). It was 30 years later in 1876 that Anton de Bary elucidated the full life cycle of the pathogen and, based on morphology of sporangia and sporangiophore characteristics, changed the pathogen name from *Peronospora* to *Phytophthora infestans* (De Bary 1876). The word *Phytophthora* means "plant destroyer". This work on a plant disease preceded work done by Louis Pasteur and Robert Koch on the germ theory of human disease.

Fig. 1.4 Sporangia of *Phytophthora infestans* on a tomato leaf

Charles Darwin

Charles Darwin studied and wrote about the pathogen after the disease struck his farm at Down House in England (Ristaino and Pfister 2016). Potatoes were the subject of some of Darwin's investigations, including studies of flowering and sexual reproduction. Darwin described the disease as a "painfully interesting subject" in letters to his cousin William Darwin Fox. Darwin had collected samples of wild potatoes from Chile during his voyages on HMS Beagle 10 years earlier, and those tubers were grow-out during the famine years to test for resistance to the disease. Unfortunately those tubers "fared exactly the same as the other kinds, having blotched in the leaf and a few tubers decayed' (Fox 1846). Darwin supported work by James Torbitt, an Irish merchant and potato breeder, from his personal funds in a search for resistance. Some of Darwin's heirloom potatoes are still grown at Down House and have potato blight.

The Spread of Potato Blight

The pathogen produces copious sporangia on leaves that can be carried by wind and rain over kilometers to spread the disease (Fig. 1.4). It is also dispersed in infected potato tubers. The blight epidemic began in 1843 in the USA, starting from the ports of New York and Philadelphia, and spread to a five-state area (Bourke 1964; Ellsworth 1843). The first appearance of the disease near the two port cities suggests an introduction via imported tubers. Two years later the disease was reported in the mainland of Europe and then spread across the English Channel into England and Ireland.

Research in my lab has focused on several "Big questions" including identifying the actual lineage that caused the famine and identifying where the pathogen came from, determining if the same lineage caused disease in the USA and Europe and Ireland, and comparing the genome to modern lineages (Martin et al. 2013; May and Ristaino 2004; Saville et al. 2016). We were the first to work with historic specimens from the actual famine era outbreaks (Fig. 1.5) (May and Ristaino 2004; Ristaino et al. 2001). Some of those specimens are housed in the Farlow Herbarium at Harvard University. We amplified three mitochondrial genes and, using SNPs from those mitochondrial genes, we were able to demonstrate that the Ia mtDNA haplotype was responsible for the first outbreaks and not the 1b haplotype as previously believed (May and Ristaino 2004; Ristaino et al. 2001; Ristaino and Hu 2009). The US-1 lineage was not the culprit behind the famine. The culprit was another SSR lineage altogether, which we subsequently named FAM-1 (Saville et al. 2016).

In 2007, The Broad Institute at the Massachusetts Institute of Technology in Boston and a large team of scientists sequenced the first full genome of *P. infestans* (Haas et al. 2009). The sequence shed light on the unusually large genome and revealed effector diversity in the pathogen. Effectors or avirulence proteins were

Fig. 1.5 Herbarium specimen of potato infected with *Phytophthora infestans* collected by J Errikson, Stockholm, Sweden in 1882

present in gene-sparse regions of the genome. The size of the genome suggested that genome expansion had occurred but further work was needed to understand that expansion. Advances in genome sequencing technology and reduced costs opened the possibility of sequencing the whole pathogen genome from historic herbarium specimens (Martin et al. 2013).

Collections from the Farlow Herbarium at Harvard, The Royal Botanic Gardens, Kew, UK, and the USDA National Fungus Collections were used in our research. The earliest samples known to be infected with *P. infestans* are housed in these important collections. Several questions of interest included whether the pathogen genome had always been large, and whether historic populations were asexual. In order to understand the evolutionary relationships among historic and more recent aggressive lineages and to determine where the famine lineage originated, comparisons of modern and historic genomes were done. We studied genome evolution with collaborators Tom Gilbert at the University of Copenhagen and Mike Martin (now at Norwegian University of Science and Technology) (Martin et al. 2013; Martin et al. 2016). At first, five historic genomes were sequenced and compared to modern-day lineages circulating in the US (US-22, US-23 and US-8) and Europe (3_A2) and the Broad sequenced strain (T30-4) (Martin et al. 2013). This study

corroborated our previous work (May and Ristaino 2004; Ristaino et al. 2001) concluding that the historic lineage was not the US-1. There was a highly supported monophyletic clade containing the historic lineages. The modern lineages were distinct and differed by over 120,000 SNPs, suggesting genome evolution and expansion with time. Many of the *Avr* genes known to be essential for virulence in modern *P. infestans* were absent in the historic lineages (Martin et al. 2013; Vleeshouwers et al. 2011) including some of the expanded set of pathogen effectors. Subsequently, we sequenced 45 additional mitochondrial genomes from historic and modern lineages (Martin et al. 2014) and results indicated that the HERB-1 (Ia) mtDNA lineage was present in Mexico and Ecuador, thus refuting the claim that the lineage was extinct (Yoshida et al. 2013). The divergence time of the HERB-1 mtDNA lineage was dated to 75 years prior to the Irish famine outbreaks (Martin et al. 2014).

We used 12-plex microsatellite analysis from a larger set of several hundred historic samples and identified the FAM-1 SSR lineage in nineteenth century USA and European samples. The same lineage caused disease on both sides of the Atlantic. Interestingly the FAM-1 lineage was found in the oldest South American samples, from Costa Rica and Colombia. The US-1 lineage was more prevalent in the mid-twentieth century. The FAM-1 lineage was present for over 100 years with a widespread distribution over six continents.

Potato Blight Today

Potato blight is not just of historic significance; it continues to cause severe disease wherever potatoes are grown (Ristaino et al. 2020). The disease is particularly devastating for smallholder farmers who do not have access to fungicides or resistant varieties. One hundred and seventy-five years after the famine, we are still trying to manage the disease. We know the pathogen moves aerially but it also moves in infected tubers and plant material. The trade and movement of potato tubers is complex and seed networks of potato can be used to understand pathogen spread (Garrett et al. 2017). Seed tubers are not always certified when they move across borders. The pathogen's polycyclic life cycle also contributes to disease spread. We have identified mefenoxam-resistant lineages. The pathogen can also shift hosts, can infect wild *Solanum* and petunias, and thus can exploit multiple niches. We have found *P. infestans* in herbarium samples from the 1850s from petunia, suggesting that the pathogen exploited alternative hosts soon after it was first described. The pathogen genome is very plastic and effector diversity contributes to increased virulence of some strains. Monoculture of susceptible varieties also contributes to disease. There are resistant varieties available but they are rarely planted on an agricultural scale. Transgenic potatoes that have resistance to the disease have been developed by the International Potato Center but the hurdles in deploying transgenic plants in the developing world are still large (Ghislain et al. 2019). Even in the USA, transgenic potatoes are not widely grown.

In 2009, a blight pandemic occurred in the USA, caused by a single lineage of US 22 (Fry et al. 2015). The lineage was spread on infected tomato transplants produced

in the south and dispersed to the northeast. The disease went undetected until it had spread to many locations, first on backyard tomatoes and then in grower fields. A team funded by a USDA NIFA grant developed the USABlight disease alert and surveillance system (Fry et al. 2013). The surveillance system sends text alerts of disease outbreaks to help growers in timing their fungicide applications. Identification of pathogen genotypes is done using 12-plex microsatellite markers and a decision-support tool was developed that uses weather data to forecast when to apply fungicides (Liu et al. 2018; Small et al. 2015; Saville and Ristaino 2019). We are also developing innovative methods to diagnose the disease rapidly using LAMP assays (Ristaino et al. 2019). This technology will allow real-time diagnosis in the field using cell-phone-based diagnostic tools that identify the pathogen in a matter of minutes, and can create outbreak maps (Li et al. 2019; Paul et al. 2019; Ristaino et al. 2019).

A global surveillance system for late blight is needed. Monitoring is heavily focused on northern Europe and the USA. Other regions of the world including Latin America (LatinBlight), China (AsiaBlight) and Africa (AfriBlight) are now beginning to collect and organize datasets and to map disease outbreaks and lineages. Funding has been a limiting factor in developing and maintaining global surveillance databases and in linking partner countries for collaborative projects. We have developed a queryable database that will enable global populations to be genotyped and identified more easily.

The exploitation of potato biodiversity for development of disease-resistant potatoes needs further research. The potato biodiversity of the Andean region is an important source of host resistance, but there is a need for more study of the biodiversity of the host and of pathogen evolution in the field, to limit occurrence of resistance-breaking strains. Host resistance is the best means for smallholders to manage blight effectively.

Climate change is expected to influence the global spread of blight; some areas may become less conducive to disease as temperatures rise. Planting climate-adapted potatoes with resistance to the disease is an important development. Continued monitoring of pathogen populations is needed to help limit disease spread and optimize management strategies, including the deployment of durable host resistance.

Huanglongbing: The Disease that Could Eliminate Orange Juice and Grapefruit from the Breakfast Table[2]

Probably the Most Devastating Citrus Disease Known

Citrus production around the world is slowly coming under threat from the disease called huanglongbing (HLB), a dialect name from China where it was first found in China, meaning yellow dragon disease (Blaustein et al. 2018). It is also called citrus greening. The threat to citrus posed by this emerging disease is serious and global,

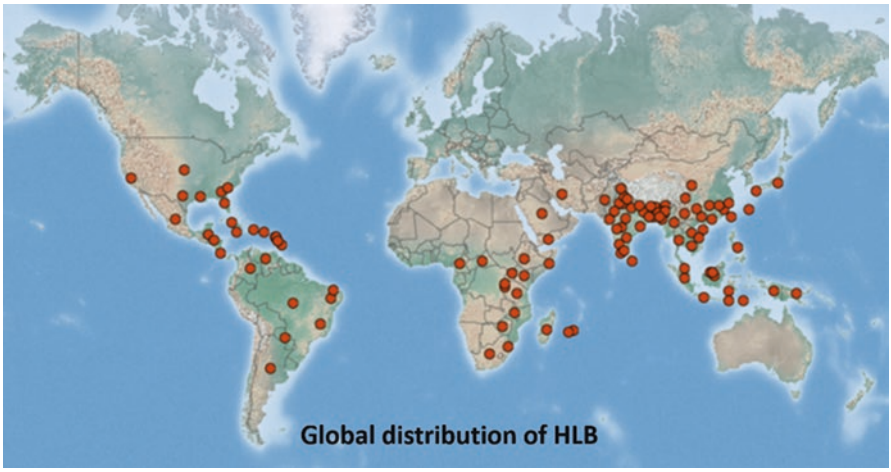

Fig. 1.6 Global distribution of HLB

potentially affecting all regions where citrus is grown. Asia and Africa are the major areas of citrus production, mostly for local consumption (Bove 2006). Brazil and Florida are the major juice producers. South Africa and Mediterranean countries are major exporters (Fig. 1.6).

HLB is probably the most devastating citrus disease known. It is a bacterial disease, unlike most other diseases of citrus. It is caused by the bacterium *Candidatus* Liberibacter asiaticus (CLas). This is a fastidious bacterium, so it cannot be studied in culture. It is quite heat-tolerant and this affects its distribution around the world. Similar diseases of citrus which may or not be classified as HLB, depending on your point of view, are caused by *Ca*. Liberibacter africanus (CLaf)) and *Ca*. Liberibacter americanus (CLam), both heat-sensitive. CLas and CLaf are quite closely related but Clam is distant from them and its emergence)in South America was unexpected.

These are vascular diseases. They are phloem-limited and they infect the whole plant so we need to be aware of what is happening underground alongside the more obvious damage to the canopy. They affect the movement of carbohydrates within the plant, by plugging up the flow of nutrients from the leaves to the roots, or vice versa depending on the season. There are probably other effects on the plant and these are the subject of active research: if we can figure out what the pathogen is doing to the plant, maybe we can stop it. Under the microscope the bacteria are pleomorphic, changing their shape from long flexuous rods to cocci, through an intermediate “lollipop” stage.

Disease Spread

The pathogen is insect-vectored by phloem-feeding specialists, about the size of a flea. The most important species is the Asian citrus psyllid, *Diaphorina citri* (Mann et al. 2018). This is widespread in South Asia and in parts of South and North America. It is not heat-sensitive. The African citrus psyllid *Trioza erytreae* is mainly found in parts of Sub-Saharan Africa. It is heat-sensitive so it tends to migrate into the forests and to higher elevations in the summer months, away from the citrus.

The global distribution of HLB has extended dramatically. First reports of unfamiliar symptoms from China and India were thought to be of a nutrient deficiency. Incidence of what could later be identified as CLas or CLaf spread through South-East Asia into the Middle East and Sub-Saharan Africa. In 2005 Brazil was hit by an unknown condition, first attributed to nutrient deficiency when neither CLas nor CLaf could be found, and then identified as CLam. Since then, CLam is gradually being out-competed and replaced by CLas.

CLas now predominates in Central and North America. The first reported outbreak in Florida was in 2005, preceded by the Asian citrus psyllid as vector in 1998. CLas was then recorded in 2009 in Lousiana, Georgia and South Carolina, in 2012 in California and Texas, and in 2017 in Alabama. The vector has now reached Arizona and Missouri.

Symptoms

Symptoms include appearance of a yellow flag on the tree, twig dieback, blotchy mottle, asymmetrical chlorosis, and misshapen fruits – the top ripening before the bottom (UF/IFAS 2020). New methods of detection using hyperspectral imaging are under investigation (Wang et al. 2019).

Effects on the crop can be drastic (Allen 2017). Orange production in Florida, mainly for juice, has plummeted in the last 5 years. In California the main incidence is on urban trees but the disease is spreading into production areas and similar effects can be expected. The disease affects the root system as well as the canopy. From the psyllid feeding point the bacterium travels rapidly towards the roots. The canopy may remain asymptomatic while 80% of the root system is lost. Control measures must therefore also reach the root system.

Disease Management

Resistance would be the preferred first line of defence. Unfortunately, few sources of resistance or tolerance have been identified in *Citrus*, and breeding a new cultivar by conventional methods may take 20 years. Genetic modification, for example for

an antibiotic trait, may offer an alternative approach. Gene editing, which does not require such exacting regulatory control is an active area of research.

Possible management strategies include:

- Vector control and rogueing of infective trees, requiring numerous applications of insecticide and regular scouting. This works best in large contiguous plantations.
- Fertilizer application, which can improve foliar symptoms but fruit drop and quality are unaffected.
- Thermotherapy: application of sufficient heat to kill the pathogen without severely damaging the host. This can be effective for potted plants but is impractical on a substantial scale.
- Vector exclusion by construction of large insect-proof screens or individual wraps for young trees.
- Antibiotics: but foliar applications do not readily enter the phloem, and trunk injection is time-consuming and costly. Novel compounds are being explored.
- Bactericidal nanoparticles that can enter phloem tissue through foliar application are being investigated.

The Future – Looking Bleak

No single strategy is sufficient for long-term management, which remains problematic. In summary, HLB is an imminent and serious threat to citrus production globally. It is likely to be aggravated by climate change. In Florida, the outlook for the citrus industry looks bleak right now.

Coffee Leaf Rust: A Persistent Threat to the Livelihoods of the People Who Produce Your Morning Cup[3]

A Devastating Disease

A coffee plantation in Costa Rica in 2012, shown in Fig. 1.7, is representative of plantations in Central America at that time. Most of the plants have lost their leaves and died of infection with coffee leaf rust, caused by the fungus *Hemileia vastatrix* "Hemileia" refers to the half-smooth character of its spores; "vastatrix" means devastating. The pathogen produces spores on the coffee plant that are released and dispersed by wind. They infect other coffee plants, entering through pores and spreading as mycelium in leaf tissue, causing lesions. From these, further spores are released, repeating the cycle – with the potential to cause an epidemic. Coffee leaf rust can cause severe losses to production; for example in Central America in 2012

Fig. 1.7 A coffee plantation in Costa Rica in 2012. Most of the plants have lost their leaves and died of infection with coffee leaf rust

losses were estimated as 20% for the region as a whole, reaching 50% in El Salvador and 75% in certain locations (Avelino et al. 2015).

This is not a new problem. In 1867 there was a severe outbreak of coffee leaf rust in the former British colony of Ceylon (now Sri Lanka). The disease was so damaging that the coffee growers switched to growing tea – and the British became tea drinkers! Coffee leaf rust arrived in the Americas in the 1970s and has been a persistent problem there ever since (Bowden et al. 1971).

Factors Favouring Disease

Outbreaks of coffee leaf rust tend to occur after periods when market prices for coffee are low, because when farm income is low farmers are less able to afford fungicides to control the pathogen, or fertilizers which enable the plant to fight off disease. The market price of coffee has fallen substantially since 2011. Climate is also a controlling factor (Bebber et al. 2016). The pathogen typically thrives at temperatures of 18–23 °C. In 1996 it was unusual to find it above 800 m, but by 2016 climate change had extended its preferred habitats so that the disease could be found up to 1500 m.

Effects on Livelihoods

Thus, we have a fungus, economics and climate change conspiring against the coffee crop, and more importantly conspiring against the people whose livelihoods depend on coffee production (Avelino et al. 2006). For example, the coffee rust outbreak in Central America in 2012–2013 was associated with a demand for labour between 16 and 32% less than in the previous year, with a huge impact on food security (Anonymous 2014). Livelihoods were impacted not only for the 1.5 million unskilled labourers who go from plantation to plantation picking the coffee berries, but also for the 240,000 smallholder farmers who depend on income to feed their families from selling the coffee they produce. In response to this case of food insecurity the World Food Programme supported 53,000 families with 8000 tons of food between July and December 2013, but that was not enough.

The US government responded to the 2012–2013 coffee leaf rust outbreak through the Feed the Future Initiative, launched in 2010 to address global hunger and food insecurity. Alongside research and technical assistance programmes, and financial assistance, we were able to develop a Coffee Rust Global Development Alliance. This is a public-private partnership between the US Agency for International Development (USAID) and World Coffee Research. World Coffee Research is “a non-profit, collaborative research and development program of the global coffee industry to grow, protect and enhance supplies of quality coffee while improving the livelihoods of the families who produce it”. It provides a channel for money from the private sector to support research projects, with matching funding from USAID. Projects are led by scientists at Texas A&M University, CATIE (Centro Agronómico Tropical de Investigación y Enseñanza) and other partners.

Options for Disease Management

In the first of these projects the *Coffee Catalog: An interactive website and printed catalog exploring 33 key varieties of Meso-America and the Caribbean* was developed by researchers who went from door to door collecting information about the coffee varieties being grown, their performance, agronomic features, disease resistance and cupping quality (World Coffee Research 2016). Some 20,000 print copies were distributed in Central America, and in 2016 it was accessed online more than 15,000 times. The project also set up regional multi-location trials of varieties from around the world in nine countries. At three locations in each country, 30 varieties were assessed for incidence of coffee leaf rust and other characters. The aim was to identify varieties that might perform better where this damaging disease occurs, especially because many of the varieties grown in Latin America have a high degree of genetic uniformity, rendering them vulnerable to new races of the pathogen, of which many have been identified.

An Arms Race Between Host and Pathogen

Since the epidemic of 2012–2013, coffee leaf rust has been responsible for the loss of 18.2 billion bags of coffee, USD 2.5 billion and 1.7 billion jobs. The crisis is still on. A resistant variety, called Lempira, which has been widely planted since the recent epidemic seems now to have lost its resistance. Plant disease can be represented as an arms race between host and pathogen. In the epidemic of 2012–2013, 13 new races of the pathogen were identified. With huge populations of the fungus and limited variation in resistance of the host there is an inevitable risk of mutations being selected that can be the cause of damaging disease. Researchers are busy working to develop varieties resistant to the current race circulating and to deploy them to plantations so smallholder farmers are able to grow coffee more sustainably.

References

Allen, G. (2017). *After a sour decade, Florida citrus may be near a comeback*. NPR February 2017. Retrieved 7 Feb 2020.

Anonymous. (2014). *Special report Central America: Coffee producer and laborer income to decline for a second consecutive year*. FEWSNET, RUTA, PROMECAFÉ. http://fews.net/sites/default/files/documents/reports/FEWS%20NET%20coffee%20rust%20shock%20report%20update_2014_02_en_0.pdf. Accessed 31 Dec 2019.

Avelino, J., Zelaya, H., Merlo, A., Pineda, A., Ordonez, M., & Savary, S. (2006). The intensity of a coffee rust epidemic is dependent on production situations. *Ecological Modelling, 197*, 431–447.

Avelino, J., Cristancho, M., Georgiou, S., Imbach, P., Aguilar, L., Bornemann, G., Laderach, P., Anzueto, F., Hruska, A. J., & Morales, C. (2015). The coffee rust crises in Colombia and Central America (2008–2013): Impacts, plausible causes and proposed solutions. *Food Security, 7*, 303–321.

Bebber, D. P., Castillo, A. D., & Gurr, S. J. (2016). Modelling coffee leaf rust risk in Colombia with climate reanalysis data. *Philosophical Transactions of the Royal Society, B: Biological Sciences, 371*, 20150458.

Berkeley, M. J. (1846). Observations, botanical and physiological, on the potato murrain. *Journal of the Horticultural Society of London, 1*, 9–34.

Blaustein, R. A., Lorca, G. L., & Teplitski, M. (2018). Challenges for managing Candidatus Liberibacter spp. (Huanglongbing disease). *Phytopathology, 108*, 424–435.

Bourke, P. M. A. (1964). Emergence of potato blight, 1943–46. *Nature, 203*, 805–808.

Bourke, P. M. A. (1993). *The visitation of god: The potato and the great famine*. Dublin: Lilliput Press.

Bove, J. M. (2006). Hunaglongbing: A destructive newly emerged century-old disease of citrus. *Journal of Plant Pathology, 88*, 7–37.

Bowden, J., Gregory, P. H., & Johnson, C. G. (1971). Possible wind transport of coffee leaf rust across the Atlantic Ocean. *Nature, 229*, 500–501.

De Bary, A. (1876). Researches into the nature of the potato-fungus – *Phytophthora infestans*. *Journal of the Royal Agricultural Society, 2*, 239–268.

Ellsworth, H. (1843). *Annual Report of Commissioner of Patents, 28th Congress, No. 150*.

Fox, W. D. (1846). Potato disease. *Gardeners' Chronicle, 40*, 661.

Fry, W. E., McGrath, M. T., Seaman, A., Zitter, T. A., McLeod, A., Danies, G., Small, I. M., Myers, K., Everts, K., Gevens, A. J., Gugino, B. K., Johnson, S. B., Judelson, H., Ristaino, J., Roberts, P., Secor, G., Seebold, K., Snover-Clift, K., Wyenandt, A., Grünwald, N. J., & Smart, D. C. (2013). The 2009 late blight pandemic in the eastern United States – Causes and results. *Plant Disease, 97*, 296–306.

Fry, W. E, Birch, P.R. J., Judelson, H. S., Grunwald, N. J., Danies, G., Everts, K. L., Gevens, A. J., Gugino, B. K., Johnson, D. A., Johnson, S. B., McGrath, M. T., . Myers, K. L., Ristaino, J. B., Roberts, P. D., Secor, G., Smart, C. D. (2015). Five reasons to consider *Phytophthora infestans* a reemerging pathogen. Phytopathology 105, 966–981.

Garrett, K. A., Andersen, K. F., Asche, F., Bowden, R. L., Forbes, G. A., Kulakow, P. A., & Zhou, B. (2017). Resistance genes in global crop breeding networks. *Phytopathology, 107*, 1268–1278.

Ghislain, M., Byarugaba, A. A., Magembe, E., Njoroge, A., Rivera, C., Roman, M. L., Tovar, J. C., Gamboa, S., Forbes, G. A., Kreuze, J. F., Barekye, A., & Kiggundu, A. (2019). Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races. *Plant Biotechnology Journal, 6*, 1119–1129.

Haas, B. J., Kamoun, S., Zody, M. C., Jiang, R. H. Y., Handsaker, R. E., et al. (2009). Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature, 461*, 393–398.

Johnson, J. F. W. (1845). *The potato disease in Scotland: Being results of investigations into its nature and origins*. Edinburgh, Scotland: William Blackwood and Sons.

Li, Z., Paul, R., Tis, T. B., Saville, A. C., Hansel, J. C., Ristaino, J. B., & Wei, Q. (2019). Noninvasive plant disease diagnostics enabled by smartphone-based fingerprinting of leaf volatiles. *Nature Plants, 5*, 856–866.

Liu, Y., Langemeier, M. R., Small, I. M., Joseph, L., Fry, W. E., Ristaino, J. B., Saville, A., Gramig, B. M., & Preckel, P. V. (2018). A risk analysis of precision agriculture technology to manage tomato late blight. *Sustainability, 10*, 3108.

Mann, M., Fattah-Hosseini, S., Ammar, E. D., Strange, R., Warrick, E. R., Sturgeon, K., Shatters, R., & Heck, M. (2018). *Diaphorina citri* nymphs are resistant to morphological changes induced by "*Candidatus Liberibacter asiaticus*" in midgut epithelial cells. *Infection and Immunity, 86*, e00889–e00817. https://doi.org/10.1128/IAI.00889-17.

Martin, M. D., Cappellini, E., Campos, P., Orlando, A. S., Orlando, L., Samaniego, J. A., Ho, S. Y., Saville, A., Dietrich, F. S., Mieczkowski, P., Heitman, J., Willerslev, E., Krogh, A., Ristaino, J. B., & Gilbert, M. P. T. (2013). Reconstructing genome evolution in historic samples of the Irish potato famine pathogen. *Nature Communications, 4*, 2172.

Martin, M. D., Ho, S. Y., Wales, N., Ristaino, J. B., & Gilbert, M. T. P. (2014). Persistence of the mitochondrial lineage responsible for the Irish potato famine in extant new world *Phytophthora infestans*. *Molecular Biology and Evolution, 31*, 1416–1420.

Martin, M., Vieira, F. G., Ho, S. Y. W., Wales, N., Schubert, M., Sequine-Orlando, A., Ristaino, J. B., & Gilbert, T. (2016). Genomic characterization of a South American *Phytophthora* hybrid mandates reassessment of the geographic origins of *Phytophthora infestans*. *Molecular Biology and Evolution, 33*, 478–491.

May, K. J., & Ristaino, J. B. (2004). Identify of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish potato famine. *Mycological Research, 108*, 471–479.

Paul, R., Saville, A. C., Hansel, J. C., Ye, Y., Ball, C., Williams, A., Chang, X., Chen, C., Gu, Z., Ristaino, J. B., & Wei, Q. (2019). Extraction of plant DNA by microneedle patch for rapid detection of plant diseases. *ACS Nano, 13*, 6540–6549.

Ristaino, J. B. (1998). The importance of archival and herbarium materials in understanding the role of oospores in late blight epidemics of the past. *Phytopathology, 88*, 1120–1130.

Ristaino, J. B., & Hu, C. (2009). DNA sequence analysis of the late-blight pathogen gives clues to the world-wide migration. *Acta Horticulturae, 834*, 27–40.

Ristaino, J. B., & Pfister, D. (2016). "What a painfully interesting subject": Charles Darwin's studies of potato late blight. *Bioscience, 62*, 1035–1045.

Ristaino, J. B., Groves, C. T., & Parra, G. (2001). PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature, 411*, 695–697.

Ristaino, J. B., Saville, A. C., Paul, R., Cooper, D., & Wei, Q. (2019). Comparison of LAMP, real-time and digital PCR for detection of *Phytophthora infestans*. *Plant Disease, 104*, 708. https://doi.org/10.1094/PDIS-06-19-1186-RE.

Ristaino, J. B., Cooke, D. E. L., Acuña, I., & Muñoz, M. (2020). The threat of late blight to global food security. Page 101–133. In A. Records & J. B. Ristaino (Eds.), *Emerging plant disease and global food security*. St. Paul: American Phytopathological Society Press.

Saville, A., & Ristaino, J. B. (2019). Genetic structure and subclonal variation of extant and recent US lineages of *Phytophthora infestans*. *Phytopathology, 109*, 614–627.

Saville, A., Martin, M., Gilbert, T., & Ristaino, J. (2016). Historic late blight outbreaks caused by a widespread dominant lineage of *Phytophthora infestans* (Mont.) de Bary. *PLoS One, 11*, e0168381.

Small, I. M., Joseph, L., & Fry, W. E. (2015). Evaluation of the BlightPro decision support system for manangemrnt of potato late blight using computer simulation and field validation. *Plant Disease, 105*, 1545–1554.

UF/IFAS. (2020). *Citrus extension: Plant pathology citrus greening.* https://crec.ifas.ufl.edu/hlb-information/greening/. Retrieved 7 Jan 2020.

Vleeshouwers, V. G. A. A., Raffaele, S., Vossen, J. H., Champouret, N., Oliva, R., Segretin, M. E., Rietman, H., Cano, L. M., Lokossou, A., Kessel, G., Pel, M. A., & Kamoun, S. (2011). Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology, 49*, 507–531.

Wang, K., Guo, D., Yao, L., Zhamf, Y., Deng, L., Xie, R., Qiang, L. V., Shilai, Y., Zheng, Y., Ma, Y., & He, S. (2019). Detection of Huanglongbing (citrus greening) based on hyperspectral image analysis and PCR. *Frontiers of Agricultural Science and Engineering, 6*, 172–180.

World Coffee Research. (2016). *Coffee varieties of MesoAmerica and the Caribbean*. https://worldcoffeeresearch.org/work/coffee-varieties-mesoamerica-and-caribbean/. Accessed 31 Dec 2019.

Yoshida, K., Schuenemann, V. J., Cano, L. M., Pais, M., Mishra, B. R. S., Lanz, R. C., Martin, F. N., Kamoun, S., Krause, J., Thines, M., Weigel, D., & Burbano, H. A. (2013). The rise and fall of the *Phytophthora infestans* lineage that triggered the Irish potato famine. *eLife, 2*, e00731. 1–25.

Chapter 2
Gone Bananas? Current and Future Impact of Fusarium Wilt on Production

Randy C. Ploetz

Abstract Banana (*Musa* spp.) is one of the world's most valuable primary agricultural commodities. Production, worth an estimated $50 billion per annum, occurs in over 130 countries. Exported fruit are key commodities in several producing countries yet they comprise less than 15% of the total annual output of 150 million metric tons. Diseases are the most significant biotic constraints for both export and smallholder production. Notably, tropical race 4 (TR4) of Fusarium wilt (aka Panama disease) affects the Cavendish subgroup, which is now responsible for 47% of all production (virtually all exported fruit and over 30% of all remaining production). The recent outbreak of TR4 in Colombia threatens future export production in tropical America, where most export production occurs. This chapter presents an overview of the disease and its causal agent, *Fusarium oxysporum* f. sp. *cubense* . Although there is a substantial positive literature on biological, chemical or cultural measures, management is largely restricted to using resistant cultivars where the pathogen has established.

Introduction

In 2003, an article in the *New Scientist* entitled "Going Bananas" discussed the current and future impact of black Sigatoka and Fusarium wilt on the global production of bananas (Pearce 2003). At the time, black Sigatoka was adequately managed with fungicides and tropical race 4 of Fusarium wilt (TR4), a Cavendish-destroying strain, had not been recognized as a serious threat. Nonetheless, the *New Scientist* article contained the following dire predictions:

- "The world's favourite fruit could disappear forever in 10 years' time."

R. C. Ploetz (✉)
University of Florida, Homestead, FL, USA
e-mail: kelly12@ufl.edu

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_2

- "We may … see the extinction of the banana as both a lifesaver for hungry and impoverished Africans and as the most popular product on the world's supermarket shelves."

In the article, Emile Frison, then Director of a global banana improvement network, was reported to have confirmed the importance of these diseases and outlined problems that are associated with producing disease-resistant replacements for susceptible bananas, notably the Cavendish subgroup. Although Emile did not predict the crop's extinction, he drew attention to the pressing need for new, disease-resistant bananas. Others who worked on banana diseases also felt that Pearce's (2003) assertion that banana would soon be extinct was an exaggeration. For example, in a response to the *New Scientist* article, Ploetz (2003) wrote:

"…it is clear that the crop will not become extinct…. The next decade will surely be a challenging one for all banana producers, but … the crop will continue to be a major food and export commodity for the foreseeable future."

Over 15 years has passed since the publication of the *New Scientist* article, yet there has not been the predicted demise, let alone reduced supply, of bananas. Global production now approaches 150 million tons of fruit per annum, 47% of which is of Cavendish. Nonetheless, the popular press loves the banana extinction story. For every rational headline that has appeared recently (e.g. "Good news: Bananas aren't going extinct. Bad news: They are in trouble") (Stephens 2018), there have been several doom and gloom headlines (e.g. "The World's Favorite Fruit is Slowly But Surely Being Driven to Extinction" (Guilford 2015) and "Bananas Facing Extinction as Deadly Fungus Spreads" (Young 2017)).

Even though the latter headlines have been used to sensationalize this story, it is clear that TR4 poses an increasingly serious threat to sustainable production of Cavendish and other susceptible bananas (Ploetz 2019). How serious a threat is TR4, when might production be reduced, and how can this problem be managed? Furthermore, by the time production is reduced will replacements for Cavendish and other susceptible bananas be available, and will they be acceptable in the marketplace?

A brief history of Fusarium wilt and the appearance and spread of TR4 is presented below. The impact and current status of TR4 is outlined, as is an overview of the disease's epidemiology and management. Neither bananas nor Cavendish will go extinct, but an eventual reckoning with TR4 is inevitable.

Fusarium Wilt of Banana

Fusarium wilt is one of the most destructive diseases of banana (Ploetz and Pegg 2000). The pathogen, *Fusarium oxysporum* f. sp. *cubense* (Foc) probably originated in Southeast Asia (Ploetz and Pegg 1997; Ploetz 2007; Stover 1962; Vakili 1965), but the disease was first recognized elsewhere. Bancroft's (1876) initial description in Australia was followed by reports from tropical America (Stover 1962), and a

dramatic increase in the number of new records occurred in the early 1900s (Ploetz 1992; Stover 1962). Currently, the disease is found in virtually all areas where banana is grown (Ploetz 2019).

The early history of this disease is associated with the first banana export trades (Stover 1962; Wardlaw 1961). Many of the first reports in a given country were on damage in export plantations of Gros Michel, the banana on which the export trades were then based, and one the disease's common names, Panama disease, refers to damage in export plantations in that country, first reported in 1890 (Brandes 1919; McKenny 1910; Ploetz 2005; Stover 1962). Throughout the banana belt, export production became increasingly difficult. Fortunately, productive Cavendish cultivars that resisted Fusarium wilt were available, and they ultimately replaced Gros Michel in the American and African trades (Stover 1962). Cavendish is essentially immune to race 1 of Foc, the cause of the Gros Michel epidemics. As Cavendish replaced Gros Michel, Fusarium wilt disappeared as a problem for the trades (Buddenhagen 1990). Black Sigatoka, caused by *Mycosphaerella* (anamorph *Pseudocercospora*) *fijiensis*, became the primary disease problem, and Fusarium wilt was forgotten as a threat to the export trades.

The resistance of the Cavendish subgroup to race 1 has been durable, and large monocultures of these cultivars have remained in production in race-1-infested soils. Although the transition to Cavendish revitalized the trades, the risk of relying on such a narrow genetic base (the Cavendish cultivars are a closely related set of clones) was recognized by those who were familiar with the Gros Michel history. Stover (1986) indicated that the export trades were "…extremely vulnerable to a new disease, especially a tropical race of Fusarium wilt that could devastate the basis of the industry – the Cavendish varieties." Shortly after this warning new plantations of Cavendish in Southeast Asia began to succumb to Fusarium wilt (Ploetz 2019). Unlike the race that affected Cavendish in the subtropics ("race 4"), TR4 was competent in good soils in the tropics, in the absence of cold temperatures or other disease-predisposing conditions. In the ensuing decades TR4 spread rapidly, first within Southeast Asia but more recently to Africa and Western Asia. To date, TR4 has been reported in Australia (Northern Territory and Queensland), China (Fujian, Guangdong, Guangxi, Hainan, and Yunnan), India, Indonesia (Bali, Halmahera, Kalimantan, Java, Papua Province, Sulawesi and Sumatra), Israel, Jordan, Laos, Lebanon, Malaysia (Peninsular and Sarawak), Mozambique, Myanmar, Oman, Pakistan, the Philippines (Mindanao), Taiwan, the United Kingdom and Vietnam (Anonymous 2019a; Butler 2013; Chittarath et al. 2017; Freshplaza 2015; Garcia-Bastidas et al. 2013; Hung et al. 2017; Molina et al. 2010; Ordonez et al. 2015; Ploetz 2019).

Although the Cavendish-based trades in the Western Hemisphere were initially unconcerned about TR4, its continued spread in the Eastern Hemisphere and the serious damage that occurred in a leading export producer, the Philippines, eventually put producers in the Western Hemisphere on high alert. In 2019, the long-feared TR4 outbreak in the west was reported in the Guajira region of Colombia (Anonymous 2019b). Confirmation of this outbreak has sent shock waves

throughout the industry in the Americas and amplified anxiety over the ultimate impact of TR4 on the export trades.

TR4 may have already caused greater losses than were experienced during the Gros Michel era. By 2006, 40,000 hectares had been affected in China (a fourfold increase from 2002) (Li et al. 2013), and a recent review indicated that "loss of hundreds of thousands of hectares of Cavendish production" had occurred in Asia (Dita et al. 2018). Reminiscent of the Gros Michel story, TR4 is spreading to new areas in China and producers are running out of arable land that is not infested, which has resulted in new production being started in neighboring countries which has subsequently succumbed as TR4 spread to these areas (note recent outbreaks in Laos, Myanmar and Vietnam). In the Philippines, only producers who have access to new (non-infested) production areas or who practice rigorous sanitation measures remain in production (G. Kema, The Netherlands, 2016, personal communication).

Race 1 also affects the Maqueño (Maia Maoli – Popoulu subgroup), Silk, Pome, and Pisang Awak cultivars, and race 2 affects cooking bananas, such as Bluggoe. Race 3, described as a pathogen of *Heliconia* spp. (tropical American banana relatives), had a minor impact on 'Gros Michel' and seedlings of *Musa balbisiana* (Waite 1963), and is no longer considered a member of f. sp. *cubense* (Ploetz and Pegg 2000).

Race 4 of Foc affects race 1 and race 2 suscepts, Pisang Mas, and Pisang Berangan, in addition to the Cavendish cultivars (Stover and Simmonds 1987; Su et al. 1977; Ploetz and Pegg 2000). Before the TR4 outbreaks in Southeast Asia, Cavendish cultivars had only been affected in the subtropics (Canary Islands, South Africa and Australia) (Ploetz et al. 1990). However, unlike subtropical race 4, TR4 affects Cavendish in the absence of predisposing factors (Ploetz 2006, 2019). Although subtropical race 4 does not affect Cavendish in the tropics, TR4 does so in the subtropics.

Epidemiology

Stover (1962) indicated that banana-free rotations were ineffective measures for managing the disease, and that Gros Michel could not be produced in previously affected plantations, due to the pathogen's long survival in soil. Twenty years was typical (Stover 1962), and 40 years has been reported (Simmonds 1966; Buddenhagen 2009). Stover (1962) suggested that chlamydospores of Foc in decayed banana tissue were responsible for its durability in infested soil.

Although chlamydospores clearly enable the pathogen to survive inhospitable conditions, it seems doubtful that they would be responsible for its decades-long persistence in the absence of a living banana host. Long-term survival as a non-pathogenic parasite of weeds may be more plausible (Ploetz 2015). Asymptomatic colonization of the roots of weeds by plant-pathogenic members of the *F. oxysporum* species complex is common (Altinok 2013; Hennessy et al. 2005; Pittaway

et al. 1999; Postic et al. 2012; Rekah et al. 2001; Waite and Dunlap 1953). Better understandings are needed for the persistence of Foc in the absence of its banana host as they may assist disease management efforts.

Foc is disseminated in diverse ways, but infected suckers are most efficient (Stover 1962). After the infectious nature of this disease was demonstrated by Brandes (1919), the trades instituted rigorous selection schemes in which suckers for new plantations were taken only from disease-free portions of fields, and those that exhibited vascular discoloration were discarded (Stover 1962). In many cases, suckers were also washed and treated with fungicides or biocides. Nonetheless, cryptically infected suckers made it past inspectors into new fields. Before tissue-culture plantlets became available, it was virtually impossible to establish pathogen-free plantations. However, even after it was possible to produce clean planting material, secondary contamination of plantations by Foc was common. For example, TR4-affected Cavendish plantations have been established with tissue-culture plantlets that were grown in infested soil (Buddenhagen 2009). In Jordan this was probably due to the use of contaminated soil when growing plantlets before transplanting into the field (Ploetz et al. 2015).

Surface waters are easily contaminated, and the use of water from contaminated rivers or ponds for irrigation is especially dangerous. Furthermore, Foc is moved in soil and on contaminated tools, farm equipment, clothes and footwear (Stover 1962). Recently, G. Kema (The Netherlands, 2016, personal communication) showed that TR4 could be moved on muddy boots contaminated in infested soils.

Any or all of these avenues can facilitate Foc movement in and around a given plantation, and other means may be possible. Meldrum et al. (2013) detected TR4 on the exoskeletons of the banana weevil, *Cosmopolites sordidus*, and suggested that the insect could be a vector or disease-predisposing agent. Aerial dissemination of Foc may also be possible since other f. spp. of *F. oxysporum* move in this manner (Elmer 2012; Timmer 1982), and macroconida/sporodochia of Foc are produced on artificially inoculated plants in greenhouse experiments (Miguel Dita and Gert Kema personal communication).

Management

There are limited options for managing Fusarium wilt of banana. The perennial nature of this disease has complicated the development of long-term measures (Ploetz 2007). Moreover, poor resistance exists in important groups of banana and technical hurdles confront those who would improve disease-susceptible cultivars (see below). The improvement of this crop has been a significant problem for as long as improvement programs have been in existence (Ortiz 2013).

In general, highly susceptible banana cultivars can be grown only if pathogen-free propagation materials are used in pathogen-free soil. Tissue-culture-derived plantlets are the most reliable source of clean material, even though they are more

susceptible to Fusarium wilt than traditional seed pieces of banana (Smith et al. 1998); they should be used to propagate this crop whenever possible. In subsistence agriculture or other situations in which the expense of tissue-culture plantlets may be an issue, they can be used to initiate disease-free nurseries to produce pathogen-free conventional seed pieces (Lule et al. 2013).

Given the ease with which Foc is spread, effective exclusion and quarantine are difficult. Nonetheless, distinguishing TR4 from other races of Foc or other members of the *F. oxysporum* species complex, the first step in mitigating new outbreaks, is possible (Ploetz 2019). A new molecular method based on the SIX effectors should enable specific detection of TR4 (Carvalhais et al. 2019). There are no effective fungicides, and long-term biological control of this disease in the field has not been demonstrated. Likewise, soil amendments and cultural and physical measures are, at best, partially or only tempozrarily effective. Although disease-suppressive soils are known, transferring this desirable trait to disease-conducive soils has not been demonstrated. In infested soil, it is usually possible to continue production only with resistant cultivars (Ploetz 2015).

Fusarium-Wilt-Resistant Cultivars

Pre-Existing Genotypes

Xu et al. (2011) conducted a cost analysis for the use of different banana genotypes . They indicated that profitable markets existed for cultivars resistant to race 1 and race TR4 in China. Which cultivars would be most profitable depended on whether plantations were infested with Foc, which race was found in infested fields, and market preferences. In infested soils in which lower rents were charged but fewer cultivars could be grown, they recommended replacing susceptible cultivars with other crops or resistant cultivars. Shorter rotations (3–5 years) and flexibility in the cultivars that could be produced were necessary in Foc-infested soil (Xu et al. 2011). Short rotations in which a banana crop is produced for only one or two cycles (before epidemic development of disease occurs) were the key to successful production of some of the first Cavendish somaclones (see below) in TR4-infested soil in Taiwan and are used to produce Gros Michel in race-1-infested soil in some areas in Latin America (Dita et al. 2018).

Dita et al. (2018) suggested that the productive life of moderately susceptible cultivars, such as Prata (Pome subgroup), could be extended with an approach broadly termed "healthy soil," in which increased microbial diversity was important. The successful use of the healthy soil approach to manage TR4 in Cavendish plantations has been suggested, but as of this writing has no empirical support.

Unfortunately, many of the world's important bananas are very susceptible to TR4, and other important bananas have unclear responses (Ploetz 2015). Better information is needed on the susceptibility of different cultivars to TR4.

Products from Conventional Breeding Programs

The first banana breeding program began in Trinidad in 1922 and was succeeded by several others. Each of these programs has faced enormous challenges (Ortiz 2013). Primitive diploids that have been used as parents by the breeding programs usually have poor agronomic and fruit traits, and introgression into advanced lines of disease resistances that they possess can take several generations (Rowe and Rosales 2000). The polyploid nature of the crop, long generation times from planting to seed production, the large size of the plant and corresponding need for large areas for hybrid evaluation, genetic abnormalities that exist in many parental lines, the need for final products to be parthenocarpic and sterile, and the low fertility of cultivars that need improvement are additional hurdles that impede progress (Lorenzen et al. 2013; Ortiz 2013; Rowe and Rosales 2000).

There is a critical need for TR4-resistant bananas that meet standards imposed by local and export markets. Currently, tolerance to TR4 is found in several bred hybrids, especially those developed by the program at the Fundación Hondureña de Investigación Agrícola (FHIA) in Honduras (Rowe and Rosales 2000). The FHIA hybrids have been widely deployed and are especially important in Cuba where they are grown without significant inputs of fertilizers and fungicides (Alvarez 1997; Alvarez and Rosales 2008). Unfortunately, only some agronomic, post-harvest and sensory standards are met by the TR4-tolerant hybrids. For example, the high-yielding dessert clones FHIA-01 and FHIA-02 had lower pulp-to-peel ratios, were not as sweet, and had lower overall consumer acceptance than the Cavendish cultivars, Grand Nain and Williams (Dadzie 1998). Thus, they could not be used by the export trades.

Somaclonal Variation

Somaclonal variation that occurs in plants that have been produced via tissue culture can be genetic or epigenetic. Although it can be useful in crop improvement, uniformity of tissue-culture plantlets can be an issue as variants occur randomly and are genetically unstable.

TR4-tolerant Cavendish somaclones were first developed by the Taiwan Banana Research Institute (TBRI) (Hwang and Ko 2004). The Giant Cavendish Tissue Culture Variants (GCTCVs) somaclones have been recurrently selected in TR4-infested fields. They have enhanced tolerance to TR4 but are not completely resistant and can usually be grown for only one or two cycles in TR4-infested sites. Poor finger and hand architecture further complicate the use of most of the somaclones by the export trades. Despite these deficiencies, the GCTCVs are currently the best TR4-tolerant alternatives for the exported Cavendish clones. Recurrently selected somaclones of Dwarf Cavendish have also been developed in Guangxi, China (Wei et al. 2016).

Genetic Modification (GM)

Genetic modification (transformation) of banana with genes from banana or other organisms has become relatively commonplace, and disease resistance is one of the most sought-after traits in genetically modified lines (Ortiz and Swennen 2014; Remy et al. 2013). There are convincing arguments for using GM to create resistant genotypes, especially when targets, such as Cavendish-like export bananas, are difficult to improve via conventional breeding (Aguilar Morán 2013).

A range of transgenes have been tested, but only short-term results from greenhouse or incubator experiments are usually reported. For example, no results are available from race 1 field trials (Subramaniam et al. 2006; Paul et al. 2011; Ghag et al. 2012), and field results for TR4 tolerance are often absent (Hu et al. 2013; Mahdavi et al. 2012; Yip et al. 2011).

Recently, Dale et al. (2017) reported transgenic TR4 resistance in Grand Nain, a Cavendish cultivar. In a TR4-infested field in the Northern Territory of Australia, a line transformed with the RGA2 gene isolated from *Musa acuminata* ssp. *malaccensis*, a TR4-resistant diploid banana, and another line transformed with a nematode-derived gene, Ced9, remained disease free for the duration of the 3-year trial. Transgene expression in the RGA2 lines was positively correlated with resistance. Endogenous RGA2 homologs were also present in wildtype Grand Nain, but were expressed at levels tenfold lower than in the most resistant transgenic line. Dale et al. (2017) suggested that the expression of RGA2 homologs might "…be elevated through gene editing, to provide non-transgenic resistance…", which would, therefore, avoid anti-GM scrutiny.

Recently, Dale's lab reported a highly efficient CRISPR/Cas9 modification system that could be used for precise gene editing in Cavendish (Naim et al. 2018). They indicated that "…an efficient gene editing platform that can manipulate endogenous disease resistance genes" was a significant step towards achieving non-transgenic resistance.

Discussion

Sensationalism has been defined as "The presentation of stories in a way that is intended to provoke public interest or excitement, at the expense of accuracy" (Oxford University Press 2018). Obviously, the idea that diseases, in particular TR4, will cause the extinction of banana has been sensationalized in the popular press. Although objective audiences would prefer accuracy, doom and gloom increases readership and sells advertising.

Although bananas will not go extinct, there will be an eventual reckoning with TR4. During the Gros Michel era, fusarium wilt of banana caused losses of US$2.4 billion (2016 conversion) (Ploetz 2019; Stover 1962). Although figures for losses from the TR4 epidemic are not available in many areas, estimates of 100,000 ha that

were recently made for China (A. Drenth, Australia, personal communication, 2016) indicate that very considerable damage has already occurred. The ultimate impact of TR4 will depend on when it is disseminated to other banana-producing regions, and whether effective control measures and better resistant lines are available once this occurs.

Despite a considerable body of literature that indicates that diverse measures can be used to manage this disease (see Thangavelu and Mustaffa 2012), there are few effective options. Most control measures that have been reported have not been tested in real-world situations in field environments, and unrealistic expectations are common (Ploetz 2015). Where the highly susceptible Cavendish cultivars are grown in TR4-infested areas it is currently necessary to produce other crops, TR4-tolerant somaclones, or resistant cultivars, such as those described by Xu et al. (2011). Unfortunately, there is still much popular mistrust of GM products, despite their proven food safety (Sanchez and Parrott 2017). Although GM bananas may not be accepted in the marketplace (Reynolds 2018), there is hope that non-GM replacements for Cavendish could be developed in the near future (Naim et al. 2018).

References

Aguilar Morán, J. F. (2013). Improvement of Cavendish banana cultivars through conventional breeding. *Acta Horticulturae, 986*, 205–208.

Altinok, H. H. (2013). *Fusarium* species isolated from common weeds in eggplant fields and symptomless hosts of *Fusarium oxysporum* f. sp. *melongenae* in Turkey. *Journal of Phytopathology, 161*, 335–340.

Alvarez, J. M. (1997). Introduccion, evaluacion, multiplicacion y diseminacion de hıbridos FHIA en Cuba. *INFOMUSA, 6*, 10–14.

Alvarez, J. M., & Rosales, F. E. (Eds.). (2008). *Identification and characterization guide for FHIA banana and plantain hybrids*. Montpellier: Bioversity International. 15 p.

Anonymous. (2019a). *Bananas at Eden Project Cornwall 18/09/19*. Accessed on 30 Oct 2019 at: https://www.shpratt.com/news/bananas-at-eden-project-cornwall

Anonymous. (2019b). Trend watch. Banana fungus goes global. *Nature, 572*, 421. https://doi.org/10.1038/d41586-019-02471-1.

Bancroft, J. (1876). *Report of the board appointed to enquire into the cause of disease affecting livestock and plants*. Queensland, 1876. Votes and Proceedings 1877, 3, 1011–1038.

Brandes, E. W. (1919). Banana wilt. *Phytopathology, 9*, 339–389.

Buddenhagen, I. W. (1990). Banana breeding and fusarium wilt. In R. C. Ploetz (Ed.), *Fusarium wilt of banana* (pp. 107–113). St. Paul: APS Press.

Buddenhagen, I. W. (2009). Understanding strain diversity in *Fusarium oxysporum* f. sp. *cubense* and history of introduction of "tropical race 4" to better manage banana production. *Acta Horticulturae, 828*, 193–204.

Butler, D. (2013). Fungus threatens top banana. *Nature, 504*, 195–196.

Carvalhais, L. C., Henderson, J., Rincon-Florez, V. A., O'Dwyer, C., Czislowski, E., Aitken, E. A. B., & Drenth, A. (2019). Molecular diagnostics of banana fusarium wilt targeting secreted-in-xylem genes. *Frontiers in Plant Science, 10*, 547. https://doi.org/10.3389/fpls.2019.00547.

Chittarath, K., Mostert, D., Crew, K. S., Viljoen, A., Kong, G., Molina, A. B., & Thomas, J. E. (2017). First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (VCG 01213/16) associated with Cavendish bananas in Laos. *Plant Disease, 102*, 449. https://doi.org/10.1094/PDIS-08-17-1197-PDN.

Dadzie, B. K. (1998). *Post-harvest characteristics of black Sigatoka resistant banana, cooking banana and plantian hybrids* (Technical Guidelines INIBAP 4). Rome: International Plant Genetic Resources Institute.

Dale, J. L., James, A., Paul, J.-V., Khanna, H., Smith, M., Peraza-Echeverria, S., Garcia-Bastidas, F., Kema, G., Waterhouse, P., Mengersen, K., & Harding, R. (2017). Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4. *Nature Communications, 8*, 1496. https://doi.org/10.1038/s41467-017-01670-6.

Dita, M., Barquero, M., Heck, D., Mizubuti, E. S. G., & Staver, C. P. (2018). Fusarium wilt of banana: Current knowledge on epidemiology and research needs toward sustainable disease management. *Frontiers in Plant Science, 9*, 1468. https://doi.org/10.3389/fpls.2018.01468.

Elmer, W. H. (2012). Biology and epidemiology. In M. L. Gullino, J. Katan, & A. Garibaldi (Eds.), *Fusarium wilts of greenhouse vegetable and ornamental crops* (pp. 11–19). St. Paul: APS Press.

Freshplaza. (2015). http://www.freshplaza.com/article/136599/QLD-Bananas-Positive-TR4-diagnosis-confirmed

Garcia-Bastidas, F. A., Ordonez, N., Konkol, J., Al Qasem, M., Naser, Z., Abdelwali, M., Salem, N. M., Waalwijk, C., Ploetz, R. C., & Kema, G. (2013). First report of *Fusarium oxysporum* f. sp.*cubense* tropical race 4 associated with Panama disease of banana outside Southeast Asia. *Plant Disease, 98*, 694.

Ghag, S. B., Singh, U. K. S., & Ganapathi, T. R. (2012). Petunia floral defensins with unique prodomains as novel candidates for development of Fusarium wilt resistance in transgenic banana plants. *PLoS One, 7*(6), e39557. https://doi.org/10.1371/journal.pone.0039557.

Guilford, G. (2015). Yes we have no bananas. The world's favorite fruit is slowly but surely being driven to extinction. *Quartz*. November 30, 2015. Accessed online on 22 October 2018, at: https://qz.com/559579/the-worlds-favorite-fruit-is-slowly-but-surely-being-driven-to-extinction/

Hennessy, C., Walduck, G., Daly, A., & Padovan, A. (2005). Weed hosts of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in northern Australia. *Australasian Plant Pathology, 34*, 115–117.

Hu, C.-H., Wei, Y.-R., & Huang, Y. H. (2013). An efficient protocol for the production of chit42 transgenic Furenzhi banana (*Musa* spp. AA group) resistant to *Fusarium oxysporum*. *In Vitro Cellular & Developmental Biology: Plant, 49*, 584–592.

Hung, T. N., Jung, N. Q., Mostert, D., Viljoen, A., Chao, C. P., & Molina, A. B. (2017). First report of Fusarium wilt of Cavendish bananas, caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (VCG 01213/16), in Vietnam. *Plant Disease, 102*, 448. https://doi.org/10.1094/PDIS-08-17-1140-PDN.

Hwang, S.-C., & Ko, W.-H. (2004). Cavendish banana cultivars resistant to Fusarium wilt acquired through somaclonal variation in Taiwan. *Plant Disease, 88*, 580–588.

Li, C. Y., Mostert, G., Zuo, C. W., Beukes, I., Yang, Q. S., Sheng, O., Kuang, R. B., Wei, Y. R., Hu, C. H., Rose, L., Karangwa, P., Yang, J., Deng, G. M., Liu, S. W., Gao, J., Viljoen, A., & Yi, G. J. (2013). Diversity and distribution of the banana wilt pathogen *Fusarium oxysporum* F. sp. *cubense* in China. *Fungal Genomics and Biology, 3*, 111. https://doi.org/10.4172/2165-8056.1000111.

Lorenzen, J., Hearne, S., Mbanjo, G., Nyine, M., & Close, T. (2013). Use of molecular markers in banana and plantain improvement. *Acta Horticulturae, 986*, 231–236.

Lule, M., Dubois, T., Coyne, D., Kisitu, D., Kamusiime, H., & Bbemba, J. (2013). *Trainer's manual. A training course on setting up and running a banana tissue culture nursery*. Ibadan: International Institute of Tropical Agriculture. 88p.

Mahdavi, F., Sariah, M., & Maziah, M. (2012). Expression of rice rhaumatin-like protein gene in transgenic banana plants enhances resistance to Fusarium wilt. *Applied and Biochemical Biotechnology, 166*, 1008–1019.

McKenny, R. E. (1910). The Central American banana blight. *Science, 31*, 750–751.

Meldrum, R. A., Daly, A. M., Tran-Nguyen, L. T. T., & Aitken, E. A. B. (2013). Are banana weevil borers a vector in spreading *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in banana plantations? *Australasian Plant Pathology, 42*, 543–549.

Molina, A. B., Williams, R. C., Hermanto, C., Suwanda, Komolong, B., & Kokoa, P. (2010). *Final report: Mitigating the threat of banana Fusarium wilt: Understanding the agroecological distribution of pathogenic forms and developing disease management strategies* (ACIAR publication ABN 34 864 955 427). Canberra: ACIAR Publication.

Naim, F., Dugdale, B., Kleidon, J., Brinin, A., Shand, K., Waterhouse, P., & Dale, J. (2018). Gene editing the phytoene desaturase alleles of Cavendish. *Transgenic Research, 27*, 451. https://doi.org/10.1007/s11248-018-0083-0.

Ordonez, N., García Bastidas, F., Laghari, H. B., Akkary, M. Y., Harfouche, E. N., al Awar, B. N., Frères, D., & Kema, G. H. J. (2015). First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 causing Panama disease in Cavendish bananas in Pakistan and Lebanon. *Plant Disease, 100*, 209. https://doi.org/10.1094/PDIS-12-14-1356-PDN.

Ortiz, R. (2013). Conventional banana and plantain breeding. *Acta Horticulturae, 986*, 177–194.

Ortiz, R., & Swennen, R. (2014). From crossbreeding to biotechnology-facilitated improvement of banana and plantain. *Biotechnology Advances, 32*, 158–169.

Oxford University Press. (2018). *Online dictionary*. Accessed on 22 October at: https://en.oxforddictionaries.com/definition/sensationalism

Paul, J.-V., Becker, D. K., Dickman, M. B., Harding, R. M., Khanna, H. K., & Dale, J. L. (2011). Apoptosis-related genes confer resistance to Fusarium wilt in transgenic 'lady finger' bananas. *Plant Biotechnology Journal, 9*, 1141–1148.

Pearce, F. (2003, January 18). Going bananas. *New Scientist, 177*(2378), 27.

Pittaway, P. A., Nasir, N., & Pegg, K. G. (1999). Soil receptivity and host pathogen dynamics in soils naturally infested with *Fusarium oxysporum* f. sp. *cubense*, the cause of Panama disease in bananas. *Australian Journal of Agricultural Research, 50*, 623–628.

Ploetz, R. C. (1992). Fusarium wilt of banana (Panama disease). In A. N. Mukhopadhyay, H. S. Chaube, J. Kumar, & U. S. Singh (Eds.), *Plant diseases of international importance* (Vol. III, pp. 270–282). Englewood Cliffs: Prentice Hall.

Ploetz, R. C. (2003, April). Yes. We won't have bananas. What realistic threats to diseases pose to banana production? *Pesticide Outlook, 14*, 62–64. https://doi.org/10.1039/b303002b.

Ploetz, R. C. (2005). Panama disease, an old nemesis rears its ugly head: Part 1, the beginnings of the banana export trades. *Plant Health Progress, 6*, 18. https://doi.org/10.1094/PHP-2005-1221-01-RV.

Ploetz, R. C. (2006). Panama disease: An old nemesis rears its ugly head. Part 2. The Cavendish era and beyond. *Plant Health Progress, 7*, 36. https://doi.org/10.1094/PHP-2006-0308-01-RV.

Ploetz, R. C. (2007). Diseases of tropical perennial crops: Challenging problems in diverse environments. *Plant Disease, 91*, 644–663.

Ploetz, R. C. (2015). Management of Fusarium wilt of banana: A review with special reference to tropical race 4. *Crop Protection, 73*, 7–15.

Ploetz, R. C. (2019). Fusarium wilt, pp 207–228. In D. R. Jones (Ed.), *Handbook of diseases of banana, Abacá and Enset*. Boston: CABI Publishing. 616 pp.

Ploetz, R. C., & Pegg, K. G. (1997). Fusarium wilt of banana and Wallace's line: Was the disease originall'y restricted to his Indo-Malayan region? *Australasian Plant Pathology, 26*, 239–249.

Ploetz, R. C., & Pegg, K. G. (2000). Fusarium wilt. In D. R. Jones (Ed.), *Diseases of banana, abaca and Enset* (pp. 143–159). Wallingford: CABI Publishing.

Ploetz, R. C., Herbert, J., Sebasigari, K., Hernandez, J. H., Pegg, K. G., Ventura, J. A., & Mayato, L. S. (1990). Importance of fusarium wilt in different banana-growing regions. In R. C. Ploetz (Ed.), *Fusarium wilt of banana* (pp. 9–26). St. Paul: APS Press.

Ploetz, R. C., Freeman, S., Konkol, J., Al-Abed, A., Naser, Z., Shalan, K., Barakat, R., & Israeli, Y. (2015). Tropical race 4 of Panama disease in the Middle East. *Phytoparasitica, 43*, 283–293.

Postic, J., Cosic, J., Vrandecic, K., Jurkovic, D., Saleh, A. A., & Leslie, J. F. (2012). Diversity of *Fusarium* species isolated from weeds and plant debris in Croatia. *Journal of Phytopathology, 160*, 76–81.

Rekah, Y., Shtienberg, D., & Katan, J. (2001). Role of the shrub *Tamarix nilotica* in dissemination of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Plant Disease, 85*, 735–739.

Remy, S., Kovács, G., Swennen, R., & Panis, B. (2013). Genetically modified bananas: Past, present and future. *Acta Horticulturae, 974*, 71–80.

Reynolds, M. (2018). Endangered species. *Wired*. November–December, pp. 108–117.
Rowe, P., & Rosales, F. (2000). Conventional banana breeding in Honduras. In D. R. Jones (Ed.), *Diseases of banana, abaca and Enset* (pp. 435–449). Oxforshire: CAB International.
Sanchez, M. A., & Parrott, W. A. (2017). Characterization of scientific studies usually cited as evidence of adverse effects of GM food/feed. *Plant Biotechnology Journal, 15*, 1227–1234.
Simmonds, N. W. (1966). *Bananas*. London: Longman.
Smith, M. K., Whiley, A. W., Searle, C., Langdon, P. W., Schaffer, B., & Pegg, K. G. (1998). Micropropagated bananas are more susceptible to fusarium wilt than plants grown from conventional material. *Australian Journal of Agricultural Research, 49*, 1133–1139.
Stephens, L. (2018). Good news: Bananas aren't going extinct. Bad news: They are in trouble. *Grist*. Dec 6, 2015. Accessed online on 22 October 2018, at: https://grist.org/food/good-news-bananas-arent-going-extinct-bad-news-they-are-in-trouble/
Stover, R. H. (1962). *Fusarial wilt (Panama Disease) of bananas and other Musa species*. Kew: Commonwealth Mycological Institute.
Stover, R. H. (1986). Disease management strategies and the survival of the banana industry. *Annual Review of Phytopathology, 24*, 83–91.
Stover, R. H., & Simmonds, N. W. (1987). *Bananas* (3rd ed.). London: Longmans.
Su, H. J., Chuang, T. Y., & Kong, W. S. (1977). Physiological race of fusarial wilt fungus attacking Cavendish banana of Taiwan. *Taiwan Banana Research Institute Special Publication, 2*, 1–21.
Subramaniam, S., Maziah, M., Sariah, M., Puad, M. P., & Xavier, R. (2006). Bioassay method for testing Fusarium wilt disease tolerance in transgenic banana. *Scientia Horticulturae, 108*, 378–389.
Thangavelu, R., & Mustaffa, M. M. (2012). Current advances in the Fusarium wilt disease management in banana with emphasis on biological control. In C. J. Cumagun (Ed.), *Plant pathology* (pp. 273–298). InTech. https://doi.org/10.5772/33775. ISBN: 978-953-51-0489-6.
Timmer, L. W. (1982). Host range and host colonization, temperature effects, and dispersal of *Fusarium oxysporum* f. sp. *citri*. *Phytopathology, 72*, 698–702.
Vakili, N. G. (1965). Fusarium wilt resistance in seedlings and mature plants of *Musa* species. *Phytopathology, 55*, 135–140.
Waite, B. H. (1963). Wilt of *Heliconia* spp. caused by *Fusarium oxysporum* f. sp. *cubense* race 3. *Tropical Agriculture, 40*, 299–305.
Waite, B. H., & Dunlap, V. C. (1953). Preliminary host range studies with *Fusarium oxysporum* f. *cubense*. *Plant Disease Report, 37*, 79–80.
Wardlaw, C. W. (1961). *Banana diseases including plantain and abaca*. New York: Wiley.
Wei, S.-L., Huang, S.-M., Wei, L.-P., Wei, D., Li, C.-S., Qin, L.-Y., Tian, D.-C., Zhang, J.-Z., Zhou, W., Long, S.-F., & Yang, L. (2016). Breeding on new banana variety Guijiao 9 resistant or tolerant to Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense,* race 4) and its high-yield cultivation technologies. *Journal of Southern Agriculture, 47*, 530–536. https://doi.org/10.3969/jissn.2095-1191.2016.04.530.
Xu, L., Huang, B., Wu, Y., Huang, Y., & Dong, T. (2011). The cost-benefit analysis for bananas diversity production in China Foc zones. *American Journal of Plant Sciences, 2*, 561–568.
Yip, M.-K., Lee, S.-W., Su, K.-C., Lin, Y.-H., Chen, T.-Y., & Feng, T.-Y. (2011). An easy and efficient protocol in the production of pflp transgenic banana against Fusarium wilt. *Plant Biotechnology Reports, 5*, 245–254.
Young, S. (2017). Bananas could face extinction due to spread of deadly fungus. One of the world's most-loved fruits is once again under threat. 18 September 2017. *Independent*. Accessed online on 22 October, at: https://www.independent.co.uk/life-style/banana-extinct-fungus-panama-spread-disease-a7953416.html

Part II
Emerging Plant Diseases and Global Food Security

Chapter 3
Plant Diseases, Global Food Security and the Role of R. Glenn Anderson

S. Rajaram and H. J. Dubin

Abstract R. Glenn Anderson was Norman Borlaug's "green fingered agricultural scientist" and humanitarian who captained the wheat revolution in India during the 1960s. Afterwards, he directed the CIMMYT International Wheat Program where he was instrumental in establishing increased wheat disease surveys, broadening of the wheat genetic diversity for adaptation, and disease resistance. He institutionalized multi-location yield and disease testing/analysis and regional breeding programs, as well as strengthening the training of young scientists. Aspects of his work and other issues are discussed in relation to present-day global food security.

Introduction

We have chosen to talk about Dr. Glenn Anderson and his achievements after the era often called the "Green Revolution". The initial Anderson lecture, given by Dr. Norman Borlaug, at the 1990 joint American Phytopathological Society / Canadian Phytopathological Society (APS/CPS) meeting presented in detail Glenn's contribution to food security in South Asia in the 1960s to early 1970s (Borlaug 1992).

The Glenn Anderson Lecture

This chapter was a contribution to the Keynote Session entitled "Emerging Plant Diseases and Global Food Security" held during the International Congress of Plant Pathology 2018, Boston, Massachusetts, USA, July 31, 2018. Some of the materials are based on unpublished observations and personal discussion of the authors with Glenn Anderson over the years 1970–1980. Wherever feasible, published citations are given.

First published in *Canadian Journal of Plant Pathology* (2019) 41, 311–317 and reproduced here with the permission of the Canadian Phytopathological Society.

Tragically, S. Rajaram died due to COVID-19 during production of this book.

S. Rajaram (Deceased)
Resource Seeds International, Toluca, Mexico

H. J. Dubin (✉)
Borlaug Training Foundation, Colorado State University, Ft. Collins, CO, USA
e-mail: hjd72@live.com

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_3

Given that 28 years have passed since that presentation, it is likely that only the "geriatric" generation of plant pathologists will recall what an outstanding humanist, scientist, and administrator Dr. Glenn Anderson was. Thus, instead of talking about rust research – past, present, and future (which will be amply covered at this meeting), we talk mostly about Glenn, the person, and his contributions to agriculture and humanity as well as science and what he might say about today's world situation. Without these special kinds of people, the world would certainly be a poorer place in all ways. In the end, all that we do is for people and a better world (Terry 1999).

Glenn was raised on a small grain and livestock farm in Ontario, Canada. His farm background served him well for his later work in South Asia. He served in the Royal Canadian Air Force University of Saskatchewan in 1955. He joined Agriculture Canada in Winnipeg where he worked on genetics of rust and smuts. He had first-hand experience in the race 15B stem rust epidemics of the 1950s and understood the disastrous effects diseases could have to wheat. He spent 11 years working on rust and smut resistance in Canada (Anonymous 1982; Higgins 1986).

Dr. Anderson was a wheat breeder *cum* pathologist who headed the Coordinated International Wheat Program for Rockefeller Foundation, Indian Council of Agricultural Research and later, after its creation, the International Maize and Wheat Improvement Center (CIMMYT in Spanish) in India, 1964–1970 (Fig. 3.1) It was the era of Malthusian fears, and famine was threatening due to drought in South Asia. He was Borlaug's General "Eisenhower" on the frontline in Asia that launched the "Green Revolution."

Fig. 3.1 Glenn Anderson et al., India, ca. 1963. Left to right—Wayne Freeman, unknown, Norman Borlaug, Glenn Anderson, S. P. Kohli. (Source: Rockefeller Foundation)

In 1971, Dr. Anderson moved to Mexico to become Associate Director of the CIMMYT International Wheat Program and in 1979, upon Norman Borlaug's retirement, he became the Director. In February 1981, while travelling in Africa for CIMMYT, he became ill, was diagnosed with leukemia, and died about 1 week later at his home in Winnipeg, Canada. It was a sudden, terrible tragedy for his family, all those who knew him, and for humanity (Borlaug 1992).

Dr. Norman Borlaug in his 1990 memorial talk at APS/CPS meeting called Dr. Glenn Anderson a "green fingered agricultural scientist in the broadest context", an excellent judge of human character with infectious enthusiasm and motivation (Borlaug 1992). He was a dedicated trainer with an ability to identify young scientists with potential. Glenn was able to stimulate those whose job it was to increase crop productivity. At the same time, he had little patience with status quo, bureaucracy, and lethargy. Dr. Borlaug concluded by saying Dr. Anderson was the "best all around wheat scientist in the world" (Borlaug 1992). It is instructive to quote a portion of Dr. Borlaug's job description for Dr. Anderson's position in India —"This type of individual must not only be a top scientist, but also a "doer". He must be able to act as a catalyst and stimulate young scientists. He must have great physical stamina and he must not accept defeat; he must fight back when things look dark. There is a great wealth of scientific talent in India that can be brought to bear upon the research problems and wheat production problems, if the right coordinator is found who can bring about this chain reaction" (Borlaug 1963). This was Glenn Anderson.

As we briefly note Dr. Anderson's contributions post "Green Revolution", it is important to realize that all of the achievements were based on genuine interdisciplinary teamwork including the generation of wheat germplasm . Dr. Anderson always emphasized that teamwork produced the best results. During the 1970s, after Dr. Anderson became Associate Director of the CIMMYT International Wheat Program, Dr. Borlaug travelled a great deal, and because of the awarding of the Nobel Prize, had to meet with government leaders, policy makers, the press, and donors among others. Dr. Anderson had a free hand and, with CIMMYT's wheat program leaders, took the initiative to continue and amplify the technical and training programs into an ever "Green Revolution" . His guidance, and distinctly caring leadership, was the key to the success of these initiatives and results from the 1970s into the 1980s and beyond. This was a major growth period for the wheat program.

Anderson's Contributions as Director of the CIMMYT International Wheat Program

Overview

Dr. Anderson, from experience, realized that to really make an impact such as that seen in South Asia and other key wheat producing areas of the lesser developed countries, he needed boots on the ground and staff placed in countries or regions.

They all had to work hand in hand with the national program scientists and administrators. He knew that well trained, young, field-oriented scientists were required, as well as improved germplasm and appropriate technology. As an all-round wheat scientist, he also wanted to document as many reliable field observations over time and space as possible, in the shortest time. This meant hiring staff, increasing germplasm flows, data collection, and analysis. Multi-location testing had started and needed to be augmented. Increasing pathology staff for screening of additional diseases besides rust was necessary as well. At the same time, laboratories such as soils, grain quality, biochemistry and cytogenetics were strengthened. Dr. Anderson honed in on all of these issues, with his program heads.

Thus, starting in the late 1960s and the decade of the 1970s, young international staff were employed in diverse disciplines and a 6-month-long field-oriented wheat training course was formalized and expanded. As the staff grew, regional programs were initiated in the Andean region and southern cone of South America, North Africa, Middle East, East Africa, and West Africa. These programs were modeled on the original country programs in India and Pakistan of the 1960s. Breeders, pathologists, agronomists as well as allied disciplines were brought in from many nations. Through the training program and international wheat seed shipments, data and note-taking were standardized in the national wheat programs in the developing world. Consequently, multi-location data could be analyzed statistically over time and space. This was a critical step forward for breeding programs over large areas. Training of young field-oriented scientists was prioritized. Additionally, a better understanding of the disease situation was clarified with the start of disease surveillance nurseries in North Africa and the Middle East as well as in South America. These were modeled on the USDA International Spring Wheat Rust Nursery (Anderson 1974). There had been FAO rust nurseries in the Near East since 1952. Dr. Anderson continually traveled to the outreach programs to give support whenever needed, as seen in Fig. 3.2 in Bangladesh, one of the great warmer climate wheat production success stories (Borlaug 1992).

The International Spring Wheat Yield Nursery was started in 1964 by Dr. Borlaug and was expanded during the late 1960s into the 1970s and finally ended in 1994 when many more ecologically focused nurseries were developed (Byerlee and Dubin 2010). Genetic research on rust resistance and types of resistance were initiated. As regional information began to flow into headquarters, studies were started on other diseases of wheat, such as foliar blights, barley yellow dwarf virus, soilborne diseases, as well as abiotic stresses, such as drought, heat, and aluminium tolerance. Concurrently, increased effort was given to breeding methodology and broadening the genetic base of the germplasm via use of winter wheat, alien species and wheat progenitors. Wide crosses, cytogenetics, statistical analysis, computerization, small grain agronomy and crop physiology were also emphasized.

Fig. 3.2 Glenn Anderson in Bangladesh wheat field, ca. 1978. (Source: Hugo Vivar, CIMMYT)

Human Resource Development in CIMMYT International Wheat Program and National Agricultural Research Systems (NARS)

As the program grew, so did the number of staff, including international senior, postdoctoral, and national researchers. The increase started in the late 1960s and peaked in the mid 1980s, and then decreased slightly for some years (Heisey et al. 2002). Disciplines that received the most increase were: breeding, pathology, training, wide crosses and cytogenetics, agronomy, and international nursery program. Staffing in support of national and regional programs in South America, in the southern cone, based in Chile, and in the Andean region, based in Ecuador, commenced. In the Middle East, the Turkish program in winter wheat was strengthened as was the program in North Africa and West Africa, located in Portugal. Increased pathology support in the region was based in Lebanon and then Egypt. The eastern and southern Africa program was opened in Kenya with a focus on rust resistance breeding. During this time, many post-doctoral fellows worked in the wheat program and many moved into new positions at CIMMYT. Of those who did not, significant numbers stayed in international development and played major roles nationally or internationally in wheat or other crops.

Drs. Borlaug and Anderson knew that well trained, young scientists were imperative to the success of the new program. Coupled with proper technology, good

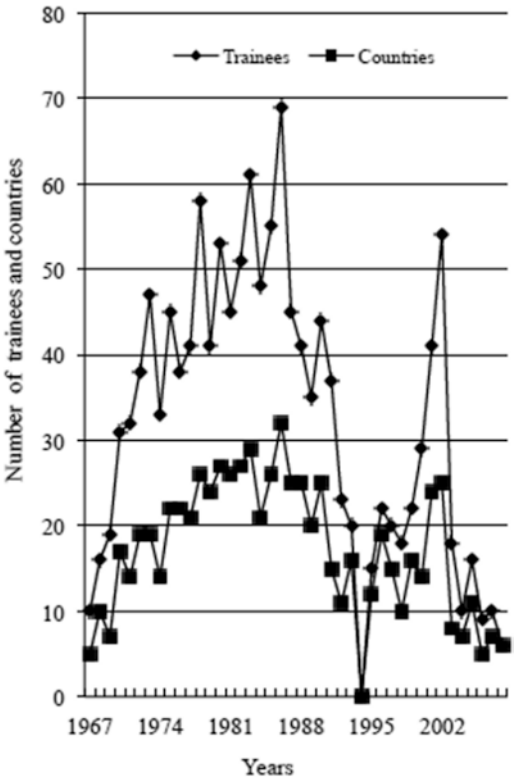

Fig. 3.3 Number of wheat program trainees in Mexico and their countries of origin, 1968–2007. (Source: Byerlee and Dubin 2010)

extension, and appropriate policy, they were the key to increased production. Dr. Anderson amplified support to the 6-month field-oriented training course to accommodate more students. By 1980, more than 50 students from 25 countries were in training (Fig. 3.3). Drs. Borlaug and Anderson even participated in rust readings as well as evening inoculations of the nurseries with the trainees. Regrettably, to this day, funding and field-oriented training is lacking for breeders at universities and international agricultural centers. The 6-month course served as a means to identify excellent students, the best of whom went on to do graduate studies. Many later became leaders in NARS like Bangladesh, Egypt, Morocco, Pakistan, Tunisia, Turkey, and others.

Breeding—Traditional, Wide Crosses, Alien Gene Introgression

During Dr. Anderson's management of the wheat program, many ideas began to bear fruit. Strategies were discussed and experiments done to guide breeders on how to obtain wheat production increases with reasonable stability over large areas. We

will touch on several of the major ones. First, a significant effort was made to enlarge the gene pool through increased number of crosses between spring wheat (SW) and winter wheat (WW) in Mexico. Winter wheat genotypes were used in the cooperating WW program at Oregon State University and CIMMYT utilized the SW in the base program for spring wheat genotypes. Increased diversity was obtained for adaptation, disease resistance, and abiotic stresses. As an example, the wheat germplasm 'Veery' group showed very significant yield increases and broader adaptation coupled with yield stability. In 'Veery', the stem rust resistance gene *Sr31*, from rye, lasted over 20 years before it was overcome in East Africa. Genetic diversity has also been increased in the advanced germplasm starting in the early 1980s (Smale et al. 2002). Increased effort was made to incorporate other alien genes into wheat from rye and wild grasses, especially for rust resistance. Initial work with synthetic hexaploid wheat was started during the late 1970s. Many useful genes, for biotic and abiotic stresses plus other useful characters, have come from these crosses (Ogbonnaya 2011).

Multi-Location Testing and Mega-Environments (MEs)

The increased distribution of wheat germplasm over large Multi-location testing areas and different environments indicated the need to classify the distinct areas in which wheat was grown. Multi-location testing Over the years, better agro-climatic data as well as biotic and abiotic stress data were accumulated. The term "mega-environments" (MEs), coined by Donald Winkelmann, an economist at CIMMYT, was used to classify a series of environments that are similar agro-climatically and biologically over large geographic areas. The use of MEs to help focus the crosses in the international breeding program is still of great importance today (Rajaram et al. 1994).

As the CIMMYT mandate expanded to cover more NARS, so did the types of experimental seed breeding nurseries shipped to cooperators (Table 3.1) and number of nurseries shipped (Fig. 3.4). The International Wheat Improvement Network (IWIN) was created to collate, analyze, and distribute the nursery information to the world wheat community (Payne 2002; Byerlee and Dubin 2010). Disease incidence over sites and years produced data that supported the ME and disease epidemiological unit classification (Rajaram et al. 1994). This was facilitated by the development of the disease surveillance and screening nurseries for Middle East and North Africa as well as for Latin America in conjunction with NARS (Anderson 1974). The great success of the IWIN has recently created a call to develop a Global Crop Improvement Network (Reynolds et al. 2018).

Table 3.1 Evolution of types of international experimental wheat seed nurseries, 1960s–1980s

Decade	Focus	Main nurseries added
1960s pre-CIMMYT and early CIMMYT [1966]	Provide best available wheat germplasm to cooperating programs with broad adaptation, high yield potential, and multiple disease resistance and test these qualities over time and space.	International Spring Wheat Yield Nursery; International Durum Yield Nursery; International Bread Wheat Screening Nursery; International Triticale Yield Nursery; International Triticale Screening Nursery.
1970s CIMMYT era	Provide high yielding, broadly adapted, daylength insensitive, multiple disease resistant germplasm. Start of spring x winter wheat breeding program. Earlier nurseries continue with additional ones as needs are determined. Specialty nurseries especially for disease resistance.	Crossing blocks; F2s Irrigated and Dryland; International Septoria Screening Nursery; Elite Spring Wheat Yield Trial; Regional Disease Trap Nursery.
1980s	As before but with additional adaptation for diverse environments. Designated as Mega-Environments. Large UNDP program on wheat for non-traditional, warmer climates.	Semi-Arid Wheat Screening Nursery; Acid Soils Wheat Screening Nursery; High Rainfall Wheat Screening Nursery; International Disease Trap Nursery; Karnal Bunt Screening Nursery.

Source: Modified from Byerlee and Dubin (2010)

Pathology and Breeding for Disease Resistance

The main thrust of the applied pathology research in the CIMMYT wheat program was to support the breeding program with increased and more durable types of resistance. However, etiology, epidemiology, and fungicide research was also concurrently carried out wherever possible. Initial work at CIMMYT, Mexico, started with rust race identification and studies of stem and leaf rust race-specific resistance genes in the program germplasm. The goal was to pyramid as many of the useful genes as possible. The work was expanded in the 1970s with increased pathology staff and excellent cooperation with rust programs in Australia, Canada, The Netherlands, UK and the USA.

Before others, Dr. Anderson was worried about stripe (yellow) rust and other, so called, minor diseases. The support of the Dutch government for a period of 10 years in training national program staff and research on stripe rust races and epidemiology was especially notable (Stubbs 1988). In the mid-1970s, the authors started research on slow-rusting resistance to leaf rust, based on observations from colleagues like Ralph Caldwell (Professor, Purdue University) as well as the literature, with the goal of finding more durable resistance (Dubin and Brennan 2009). These studies led to incorporation of several slow-rusting resistance genes in the CIMMYT germplasm (Rajaram et al. 1988; Singh et al. 2004). Later, genetic studies identified these genes and significant progress has been made on the unique modes of action of this type of durable resistance and its introgression into wheat germplasm (Krattinger

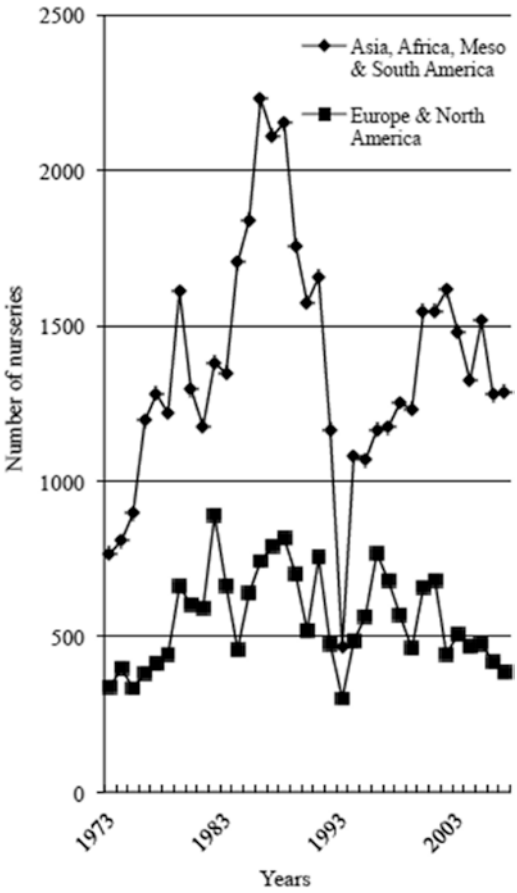

Fig. 3.4 Number of international experimental wheat seed nurseries shipped in 1970s–2000s. (Source: Byerlee and Dubin 2010)

et al. 2009). Gene introgression, from other grass species into wheat, for rust resistance, has also been very useful for longer-lasting resistance but also has been short-lived in many cases. Multi-location testing and use of "hot spot locations" where the virulence spectrum of rusts was diverse helped to produce longer-lasting resistance combinations. Key areas continue to be the Kenyan and Ecuadorian highlands for stem and stripe rust, respectively (Rajaram et al. 1988) .

Global Food Security and Glenn Anderson

Dr. Anderson had realized in the early 1970s that India's population would continue to increase rapidly and could reach 1.5 billion people at some future date. He also believed that, if natural resources like soil, water and inputs like germplasm and fertilizer were well managed and sufficient investments were provided in scientific

research, as well as sound government policy, India could remain self sufficient in food production.

Currently, India's population is 1.3 billion and is projected to increase to 1.7 billion by 2050. In the late 1960s, the population growth rate was 2.1% and in 2018 it is estimated to be 1.1%, a remarkable decrease. However, India is still expected to surpass China's population by 2028 (United Nations 2018).

The question we pose is –what would Glenn do in the current and future global scenario? One of the most pressing issues today for global food security is the estimated continuing world population increase from 7.4 billion in 2015 to 9.8 billion by 2050. Today, more than 800 million still go hungry. Africa is especially critical in this regard. Although we see population increases as one of the key factors in food security, others consider population less important today than previously, based on the decrease in wheat consumption in recent years except in areas that do not produce wheat. Stabilizing crop yields in the face of climate change and biotic and abiotic stresses is critically important as well. Global warming will be most devastating for agricultural production in parts of the tropical and sub-tropical countries including South Asia, southern Africa, parts of South America and the Caribbean. The frequency of high temperatures, freezing, droughts, flooding and salinity will have disastrous consequences for food production especially grains, roots, fiber and forage crops.

How would Glenn have reacted to these issues? He was keenly aware of the "population monster" and of many other issues that still exist today that he faced, like policy infrastructure, agricultural research (especially the yield gap), education, harvest losses, and others. Less obvious at that time were issues such as overuse of inputs, social/gender issues, and urbanization. His overriding focus was alleviating hunger in time of famine. Climate change and its disastrous effects on the natural resource base were not yet on the horizon.

Some of the problems noted above that were immediate for Dr. Anderson in India, in the 1960s, were drought, water availability, poor fertility of the Indian soils and on top of that a huge government bureaucracy. His response was to develop a strong, multidisciplinary and coordinated team of geneticists, plant breeders, agronomists, pathologists, soil scientists, farmers, seed producers, and policy makers from different institutions in the states across India. The result was the "Green Revolution".

We believe that the solution for the food security problems worldwide is similar. It will require increased cooperation and coordinated efforts on the part of the international community in relation to the factors noted, in order to provide adequate food for the coming generations.

Acknowledgements We gratefully acknowledge the Canadian Phytopathological Society and the International Congress of Plant Pathology 2018 for the invitation and financial support to present The Glenn Anderson Lecture, and for publication of the presentation. We also thank Brent McCallum for his help. We thank J. P. Gustafson and M. A. McMahon for their comments and inputs on the manuscript. We also are grateful to D. Byerlee and R. DePauw for their role as reviewers. Any inadvertent errors in the manuscript are the sole responsibility of the authors.

References

Anderson, R. G. (1974). *A second look at the green revolution. The 1974 Klinck lecture* (Agricultural Institute of Canada. CIMMYT translation and reprint no. 21). Mexico: CIMMYT.

Anonymous. (1982). R. Glenn Anderson. International Agronomy Award. *Agronomy Journal, 74*, 220–221.

Borlaug, N. E. (1963). *Wheat production problems in India* (Rockefeller Foundation trip report) (p. 27). Mexico: CIMMYT.

Borlaug, N. E. (1992). World food security and the legacy of Canadian wheat scientist R. Glenn Anderson. *Canadian Journal of Plant Pathology, 14*, 254–266.

Byerlee, D., & Dubin, H. J. (2010). Crop improvement in the CGIAR as a global success story of open access and international cooperation. *International Journal of the Commons, 4*(1), 452–480.

Dubin, H. J., & Brennan, J. P. (2009). *Combating stem and leaf rust of wheat: Historical perspective, impacts and lessons learned* (IFPRI discussion paper 910) (pp. 23–24). Washington, DC: International Food Policy Research Institute.

Heisey, P. W., Lantican, M. A., & Dubin, H. J. (2002). *Impacts of international wheat breeding research in developing countries, 1966–1997* (pp. 11–12). Mexico: CIMMYT.

Higgins, V. J. (1986). CPS and APS sponsor Glenn Anderson lecture on security of the world food supply. *Plant Disease, 74*, 200.

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini, E., Selter, L. L., & Keller, B. (2009). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science, 323*(5919), 1360–1363.

Ogbonnaya, F. C. (2011). Development, management and utilization of synthetic hexaploids in wheat management. In A. P. Bonjean, W. J. Angus, & M. van Ginkel (Eds.), *The world wheat book: A history of wheat breeding* (Vol. 2, pp. 823–843). Paris: Lavoisier.

Payne, T. S. (2002). *The international wheat improvement network (IWIN) at CIMMYT*. http://libcatalog.cimmyt.org/Download/cim/448549.pdf. Accessed on 29 May 2018.

Rajaram, S., Singh, R. P., & Torres, E. (1988). Current CIMMYT approaches to breeding wheat for rust resistance. In N. W. Simmonds & S. Rajaram (Eds.), *Breeding strategies for resistance to rusts of wheat* (pp. 101–118). Mexico: CIMMYT.

Rajaram, S., van Ginkel, M., Fischer, R. A. (1994). CIMMYT's wheat breeding mega-environments (ME). In *Proceedings of the 8th International Wheat Genetics Symposium*, July 19–24, Beijing, China. pp. 1101–1106.

Reynolds, M. P., Braun, H. J., Cavalieri, A. J., Chapotin, S., Davies, W. J., Ellul, P., Feuillet, C., Govaerts, B., Kropff, M. J., Lucas, H., Nelson, J., Powell, W., Quilligan, E., Rosegrant, M. W., Singh, R. P., Sonder, K., Tang, H., Visscher, S., & Wang, R. (2018). Improving global integration of crop research: Field laboratories in realistic crop environments are needed. *Science, 357*(6349), 359–360.

Singh, R. P., Rajaram, S., Saini, R. G., Huerta-Espinosa, J., & William, M. (2004). Genetics of host-pathogen interaction and breeding for durable resistance. In H. K. Jain & M. C. Kharkwal (Eds.), *Plant breeding – Mendelian to molecular approaches* (pp. 145–166). New Delhi: Narosa.

Smale, M., Reynolds, M. P., Warburton, M., Skovmand, B., Trethowan, R., Singh, R. P., Ortiz-Monasterio, I., & Crossa, J. (2002). Dimensions of diversity in modern spring bread wheat in developing countries from 1965 to 2000. *Crop Science, 42*, 1766–1779.

Stubbs, R. W. (1988). Pathogenicity analysis of yellow (stripe) rust of wheat and its significance in a global context. In N. W. Simmonds & S. Rajaram (Eds.), *Breeding strategies for resistance to rusts of wheat* (pp. 23–38). Mexico: CIMMYT.

Terry, E. R. (1999). Ecological stability and crop protection: A case for investment in technological alternatives. *Plant Pathology, 48*, 679–688.

United Nations Population Estimates and Projections. (2018). http://worldpopulationreview.com/countries/india-population/. Accessed 29 May 2018.

Chapter 4
Optimising Reactive Disease Management Using Spatially Explicit Models at the Landscape Scale

Frédéric Fabre, Jérôme Coville, and Nik J. Cunniffe

Abstract Increasing rates of global trade and travel, as well as changing climatic patterns, have led to more frequent outbreaks of plant disease epidemics worldwide. Mathematical modelling is a key tool in predicting where and how these new threats will spread, as well as in assessing how damaging they might be. Models can also be used to inform disease management, providing a rational methodology for comparing the performance of possible control strategies against one another. For emerging epidemics, in which new pathogens or pathogen strains are actively spreading into new regions, the spatial component of spread becomes particularly important, both to make predictions and to optimise disease control. In this chapter we illustrate how the spatial spread of emerging plant diseases can be modelled at the landscape scale via spatially explicit compartmental models. Our particular focus is on the crucial role of the dispersal kernel – which parameterises the probability of pathogen spread from an infected host to susceptible hosts at any given distance – in determining outcomes of epidemics. We add disease management to our model by testing performance of a simple "one off" form of reactive disease control, in which sites within a particular distance of locations detected to contain infection are removed in a single round of disease management. We use this simplified model to show how ostensibly arcane decisions made by the modeller – most

Electronic Supplementary Material: The online version of this chapter (https://doi.org/10.1007/978-3-030-57899-2_4) contains supplementary material, which is available to authorized users.

F. Fabre
UMR SAVE, INRAE, Bordeaux Sciences Agro, Villenave d'Ornon, France
e-mail: frederic.fabre@inrae.fr

J. Coville
UR BIOSP, INRAE, Domaine Saint Paul, Avignon Cedex 9, France
e-mail: jerome.coville@inrae.fr

N. J. Cunniffe (✉)
Department of Plant Sciences, University of Cambridge, Cambridge, UK
e-mail: njc1001@cam.ac.uk

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_4

notably whether or not the underpinning disease model allows for stochasticity (i.e. randomness) – can greatly impact on disease management recommendations. Our chapter is accompanied (*see* Supplementary Material) by example code in the programming language R available via an online repository, allowing readers to run the models we present for themselves.

[1]Introduction

Diseases in crop plants can significantly impact food security (Strange and Scott 2005), as well as production costs of food (Oerke 2006; Savary et al. 2019) and timber (Pimentel et al. 2005). Diseases in natural environments can affect a wide range of ecosystem services (Boyd et al. 2013). Understanding when and where plant disease outbreaks are likely to occur – as well as how outbreaks can be managed effectively – is therefore imperative (Cunniffe et al. 2015a, 2016).

Many plant pathogens are extremely well established, regularly causing disease in any given location with depressing predictability, at least in the absence of crop protection. However, other pathogens – or new strains of existing pathogens – are actively spreading, leading to disease-induced losses in new regions. Our focus here is such "emerging epidemics" (Almeida 2018), since control then has a particularly strong spatial component.

A number of high-profile emerging epidemics are currently threatening crop production worldwide, including citrus canker (Gottwald et al. 2002) and huanglongbing (Gottwald 2010) in Brazil and the United States; cassava brown streak virus (Legg et al. 2011) and maize lethal necrosis in East Africa (Mahuku et al. 2015); Race TR4 of Panama disease in Mozambique (Ordonez et al. 2015); coffee leaf rust in South America (Talhinhas et al. 2017); novel races of wheat stem rust in Africa and the Middle East (Singh et al. 2011); and olive decline (caused by *Xylella fastidiosa*) in Europe (Martelli 2016).

Non-native invasive pathogens in forest environments can also lead to profound population, community, ecosystem and economic impacts (Stenlid et al. 2011; Roy et al. 2014). A prominent historical example is the virtual eradication of American chestnut from the Eastern United States in the early 1900s due to chestnut blight, caused by the ascomycete *Cryphonectria parasitica* (Freinkel 1997). Another is Dutch elm disease – caused by the beetle-vectored fungal pathogen *Ophiostoma novo-ulmi* – which decimated elm populations in the United States and large areas of Western Europe in the 1960s and 1970s (Gibbs 1978). Contemporary examples include *Phytophthora ramorum*, the causal agent of sudden oak death in the United States (Rizzo et al. 2005) and ramorum disease in the United Kingdom (Brasier and

[1] See also Online Resource to this Chapter with details of the model, additional figures and video animation.

Webber 2010), as well as ash dieback (caused by *Chalara fraxinea*) across almost all of Europe (Timmerman et al. 2011).

Factors implicated in the long-distance spread of plant pathogens are numerous. Many plant pathogens, particularly those fungi that cause rust and mildew diseases, are very well adapted to spread aerially over extremely long distances (Brown and Hovmøller 2002). Long-distance dissemination linked to the water cycle and circulation of tropospheric air masses has been described for bacteria (Morris et al. 2013). Geographical ranges of pathogens – and host plants and vectors – are changing, driven in part by climate change (Bebber 2015). Altered patterns of trade and travel have also caused rates of pathogen introduction to increase (Brasier 2008). New strains of pathogens resistant to fungicides – or virulent on previously resistant crop varieties – can also spread widely (Fry and Goodwin 1997). This is particularly promoted by crop monocultures relying on single resistance genes, or combinations of resistance genes. In such cases, if even one plant becomes infected, whole fields or even regions can rapidly be lost to disease (Brown 1995).

In facing the challenge posed by emerging epidemics, mathematical modelling can play a major role, particularly in designing and optimising management strategies (e.g. Cunniffe et al. 2015b; Thompson et al. 2018; Martinetti and Soubeyrand 2019). This is all the more important when frequent long-distance pathogen dispersal events mean that management over large spatial scales (i.e. the "landscape" scale) must be considered (Plantegenest et al. 2007; Gilligan 2008). Modelling offers an alternative to traditional approaches based on expert opinion, and is greatly facilitated by the progress made in the last decade in computational biology. Models provide a rational basis to integrate what is known about a pathogen with what can be learnt from early patterns of spread, while allowing for that which is not very well characterised but can be plausibly inferred from expert knowledge. The utility of such a model then lies in its ability to make predictions of future spread, which in turn can inform strategies to optimise disease detection and management (Gilligan 2008). At the same time, however, it is imperative to recognise that a model is not a magic bullet, at least until it has been properly parameterised and validated.

For emerging epidemics, questions of practical interest are often inherently spatial. Given a certain level of resource to be expended on sampling for disease, which regions should be prioritised for surveillance? How to tailor surveillance strategies to landscape features? Once the disease has been detected, where will disease spread to next? How long will that take? How should local management be done? Which regions should be prioritised for control? Is control likely to be a success? How robust are management strategies to changing landscape contexts? These questions clearly require models to include a spatial component. Providing an introduction to the most common framework by which such questions are answered – the spatially explicit compartmental model – is one of the purposes of this chapter. An explicit intent is to make code available to allow readers to run the model(s) for themselves.

Emerging plant diseases are most often managed reactively, i.e. sites within a particular distance of locations detected to contain infection are targeted for control. This is often host removal, particularly for high-value crops such as fruit trees, but in principle could also be chemical treatment. The rationale is to treat or remove

locations that are likely to be infected without yet showing symptoms. Taking just three real-world examples, such management is currently in progress for olive quick decline in Italy (Martelli 2016), wheat blast in Bangladesh (Callaway 2016) and sudden oak death in Oregon, United States (Peterson et al. 2015). It was also the basis of the decade-long attempt to eradicate sharka, a disease of prunus trees in several countries worldwide (Rimbaud et al. 2015), as well as citrus canker from Florida following its first introduction in 1995 (Gottwald et al. 2002). In the case of citrus canker, the attempt to eradicate was only stopped after being judged to have failed following removal of over 10 million citrus trees, at an estimated cost of over 1 billion dollars (Irey et al. 2006).

Such high-profile failures in control have led to a high interest from mathematical modellers. There is now a very good understanding of factors promoting the success of reactive control, including in models parameterised to the spread of particular pathogens (Cunniffe et al. 2015b; Parnell et al. 2009, 2010; Thompson et al. 2016a). More recent work has also considered how the controls can be extended to include more epidemiology, for example by including a notion of the risk of infection (Hyatt-Twynam et al. 2017; Adrakey et al. 2017), or by making controls more elaborate using tools from optimal control theory (Bussell et al. 2019). Other work has shown how reactive control can be made to be successful at very large scales even for a very well established epidemic when there is a limited budget (Cunniffe et al. 2016). However, what has not yet been tested explicitly is the effect of model structure – and in particular whether the underpinning epidemic model is deterministic or stochastic – on the extent to which a mathematical model can be used to generate the types of prediction needed to inform reactive control. Doing this, as well as providing an introduction to the theory underpinning dispersal kernels and providing a reference implementation of models in R for use by the reader, is the contribution we offer in this chapter.

Overview of the Theory of Dispersal Kernel and the Current Knowledge of Dispersal Kernels in Plant Pathology

Mathematical Classification of Dispersal Kernels

Many questions in both theoretical and applied epidemiology are inherently spatial. For many pathogens, transmission depends on contact between susceptible and infected individuals. In the case of sessile hosts – including plants – this in turn often depends on the distances between pairs of hosts. The movement of pathogen dispersers (e.g. spores, propagules, vectors: henceforth “inoculum”) can then be described by a location dispersal kernel (Nathan et al. 2012). In this context it represents the statistical distribution of the location of the inoculum after dispersal from a point source. In two-dimensional space, the dispersal kernel can be defined as the probability density $J(x, y)$ that a propagule emitted from a point source at (0,

0) is deposited in position (x, y) (which is at a distance of $r = \sqrt{\left(x^2 + y^2\right)}$). Several families of location dispersal kernels are classical in ecology (Klein et al. 2006; Nathan et al. 2012). Although it is possible to imagine a number of deviations from this ideal in the real-world, kernels as used in models are almost always isotropic, meaning that transmission probabilities decay with distance uniformly along all radial directions.

Dispersal kernels are firstly defined by their scale, which can be taken to correspond to the mean dispersal distance. Here, we consider two families of kernels. The first is the exponential-power kernel, defined in two dimensions as

$$J_{EP}\left(x,y\right) = \frac{\tau_{disp}}{2\pi\alpha^2\Gamma\left(2/\tau\right)}\exp\left(-\left(\frac{r}{\alpha}\right)^{\tau_{disp}}\right),$$

with τ_{disp}, $\alpha > 0$ and so scale (i.e. mean dispersal distance) $\mu_{disp} = \alpha\Gamma(3\tau)/\Gamma(2\tau)$, and where the normalising constant follows from integration over all of two-dimensional space. The second is the (inverse) power-law, defined in two dimensions as

$$J_{PL}\left(x,y\right) = \frac{\left(\tau_{disp}-2\right)\left(\tau_{disp}-1\right)}{2\pi\alpha^2}\left(1+\frac{r}{\alpha}\right)^{-\tau_{disp}},$$

Dispersal kernels can be further defined by their shape, which informs in particular the "fatness" of their tails. This characterises the magnitude and frequency of long-distance dispersal events, defined, for example, as the proportion of dispersal events exceeding a given distance (e.g. the value of quantile 99% of the underpinning probability distribution for distances from the source). The shapes of several kernels sharing the same mean dispersal distance $\mu_{disp} = 80$ are illustrated in Fig. 4.1a. Clearly, kernel shapes drastically impact the relative proportion of long-distance dispersal events. Shapes of dispersal kernels also differ markedly close to the origin, and in particular exponential-power kernels with $\tau_{disp}<1$ are typically very strongly peaked.

The "fatness" of the kernel tail can be used to categorise kernels in a binary fashion (Mollison 1977). When, at relatively large distance, the shape of the tail decreases less slowly than exponential distribution, or equally slowly, a kernel is termed "short-tailed" or "thin-tailed" (Klein et al. 2006). This is the case for the exponential-power kernels whenever $\tau_{disp}>1$. Certain thin-tailed variants of the exponential-power kernel are very well known in their own right, being sufficiently well used to merit a specific name, including the Gaussian ($\tau_{disp}=2$) and the exponential kernels ($\tau_{disp}=1$). We note the latter kernel actually defines the frontier between thin- and fat-tailed kernels.

In contrast, if the probability of dispersal decreases more slowly than an exponential distribution at long distances from the source, kernels are termed "long-tailed" or "fat-tailed". Long-distance dispersal events are then more frequent than with an exponential kernel that shares the same mean dispersal distance. This is the

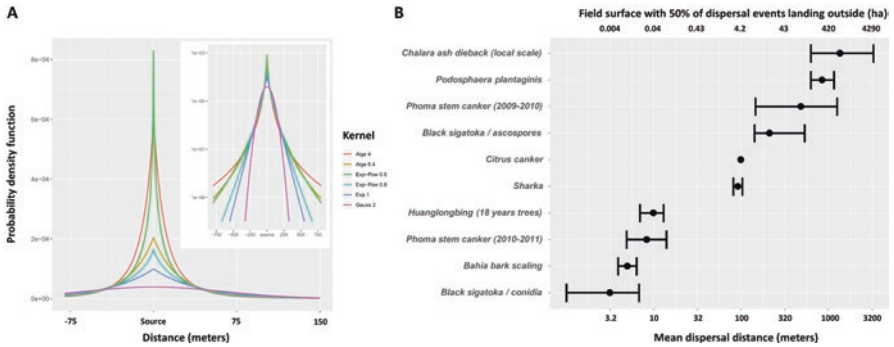

Fig. 4.1 Current knowledge of dispersal kernels in plant pathology. (**a**) Six dispersal kernels obtained within the power-law and the exponential-power distribution families. All have the same mean dispersal distance (here μ_{disp} = 80 m) and differ only by the derived parameter τ_{disp} that controls the weight of the tail. In particular we show a thin-tailed Gaussian dispersal kernel (τ_{disp} = 2, quantile 99% = 194 m), an exponential kernel (τ_{disp} =1, quantile 99% = 265 m), two fat-tailed exponential–power kernels (τ_{disp} = 0.8, quantile 99% = 300 m; τ_{disp} = 0.5, quantile 99% = 404 m) and two very-fat-tailed power-law kernels (τ_{disp} = 4, quantile 99% = 639 m; τ_{disp} = 6.4, quantile 99% = 404 m). (**b**) Graphical summary of our literature review which uncovered the mean dispersal distance for eight plant pathogens. For each pathosystem, the point displays the mean (or median) and the horizontal line the extent of a 95% confidence interval (i.e. the range between the 2.5% and 97.5% quantiles of the full distribution of outputs). The secondary x-axis along the top of the figure displays, for each mean dispersal distance on the principal x-axis, and assuming that infectious propagules are emitted according an exponential kernel from plants randomly scattered inside the field, the field area (in hectares) for which 50% of the propagules produced land outside field bounds (see text). References to the particular studies used are summarised in Table 4.1

case for both the exponential-power kernels with τ_{disp}<1 and for all power-law kernels. Fat-tailed kernels can be further distinguished depending on whether they are "regularly varying" (e.g. power law kernels) or "rapidly varying" (e.g. exponential-power kernels) (Klein et al. 2006). Mathematically, it implies that power-law kernels decrease even more slowly than any exponential-power function. Biologically this means that fat-tailed exponential-power kernels display rarer long-distance dispersal events than power-law kernels. As we shall see below, this distinction potentially has important implications for disease control.

Current Knowledge of the Dispersal Kernel in Plant Pathology

Characterising dispersal kernels of plant pathogens can be very challenging. The most obvious methods require observations of inoculum dispersal patterns over large-scale ranges of distances (Kuparinen et al. 2007). Dispersal kernels can also be inferred by fitting epidemic models to disease spread data, but this requires detailed spatially explicit disease data, again over wide spatial scales and often finely time-resolved, as well as sophisticated statistical analysis (Soubeyrand et al.

Table 4.1 Review of dispersal kernels obtained for plant pathogens

	Size of the experimental design/site	Kernels considered	Anisotropy	Mean/Median (in meters)	Tail fatness	References
Sharka, plum pox virus transmitted by *Aphis gossypii*	5.6 km × 4.8 km (553 orchards)	Beta-weighted mixture of exponentials (BWME) kernel[a]	No	Median [CI 95%] 92.8 [82.6–104]	Fat-tailed kernel	Pleydell et al. (2018)
Black Sigatoka, *Mycosphaerella fijiensis*/ Ascospores	Trap plant network of 1 km radius	Exponential-power	Yes	Mean [CI 95%] 213 [144–542] D1000 14721 [2134–184267] Dinf [(b)]	Fat-tailed kernel	Rieux et al. (2014)
Black Sigatoka, *Mycosphaerella fijiensis*/ conidia	Trap plant network of 25 m radius	Exponential-power	Yes	Mean [CI 95%] 3.15 [1.01–6.78] D25 [(b)] 6.12 [2.79–8.16] Dinf [(b)]	Thin-tailed kernel	Rieux et al. (2014)
Powdery mildew, *Podosphaera plantaginis*	50 km × 70 km (4000 meadows)	Exponential	Yes	Mean [CI 95%] 860 [640–1180]	Thin-tailed kernel (but a single kernel tested)	Soubeyrand et al. (2009)
Phoma stem canker, *Leptosphaeria maculans* Ascospores	2.5 km × 2 km	Exponential-power	No	Mean [CI 95%] (years) 490 [147–1273] (2009–2010) 8.4 [4.9–14.1] (2010–2011)	Fat-tailed kernel	Bousset et al. (2015)

(continued)

Table 4.1 (continued)

	Size of the experimental design/site	Kernels considered	Anisotropy	Mean/Median (in meters)	Tail fatness	References
Bahia bark scaling of citrus, little is known of a putative pathogen	420 m × 212 m	Exponential kernel	No	Median [CI 95%] 5 [3.92–6.42]	Thin-tailed kernel (but a single kernel tested)	Cunniffe et al. (2014)
Chalara ash dieback, *Hymenoscyphus fraxineus*	Local: traps from 0 m to 800 m Regional: 15 transects from 40 km to 140 km	Inverse power law, Gaussian	Yes (at regional scale)	Mean [CI 95%] 1380 [640-3320] "Local scale" 2560 [80–7650] "Regional scale"	Fat-tailed kernel	Grosdidier et al. (2018)
Huanglongbing, bacteria (*Candidatus* Liberibacter) transmitted by psyllids	3.5 km × 2.4 km	Exponential, power-law	No	Mean [CI 95%, based on sd = 1.5] 5 [2–8] for 5 years-old trees 10 [7–13] for 18 years-old trees	Thin-tailed kernel	Parry et al. (2014)
Citrus canker, *Xanthomonas axonopodis*	4 sites from 1 km² to 4 km²	Exponential, Cauchy[c]	No	Mode 50 to 120	No significant difference between exponential and Cauchy kernel when primary infection is accounted for	Neri et al. (2014)

[a]The BWME kernels provide close approximations to exponential-power and power-law kernels for a wide range of parameters tested by Pleydell et al. (2018) while making the method used for parameter estimation easier

[b]Two estimates are provided, with and without considering the tail of the kernel at distances higher the radius of the experimental design

[c]The Cauchy kernel used by Neri et al. (2014) is closely related to the power-law kernels we consider

2009). Consequently, relatively few studies report fitted parameters for dispersal kernels of particular plant pathogens.

Our (non-systematic) literature review identified only eight studies reporting dispersal kernels for plant pathogens that used data gathered in experimental designs extending over regions in excess of 1 km^2. These mostly concerned fungal pathogens, but a few focused on diseases caused by viruses and bacteria (Table 4.1, Fig. 4.1b). Mean dispersal distances ranged from a few meters up to 1 kilometer, with several estimates around 100 m. However, the forms of the dispersal kernel varied, making direct comparison difficult. A study by Filipe et al. (2012) on sudden oak death in California which was conducted on a large area was not retained in what follows, since a full description of the fitted dispersal kernel was not given – details of short-distance (i.e. within 125 m) dispersal not being required by their underlying cell-based model.

To provide a more intuitive basis for comparison, as well as to highlight the importance of the landscape scale when addressing both basic and applied epidemiological questions, we used an individual-based approach to simulate the dispersion of propagules from a focal square field. The question is related to in-depth studies of pollen dispersion in agricultural landscapes (Lavigne et al. 2008). In all cases for computational simplicity we used the exponential kernel. Its single parameter defines (in particular) the mean dispersal distance. Assuming that infectious propagules are emitted from plants randomly scattered inside the focal infected field, we assessed, for increasing mean dispersal distances, the field area for which 50% of the pathogen propagules produced would land outside the field considered. These values are reported on the secondary x-axis of Fig. 4.1b. A mean dispersal distance of 100 m implies that 50% of the pathogen propagules produced will land outside a field of a surface of 4.2 hectares (side length 205 m). This is higher than 2.7 ha, the median field size cultivated in France for the main cash crops (excluding market gardening, arboriculture, viticulture) (Barbu, pers. comm., data for 2014).

Seven of the eight studies we identified used model selection approaches to distinguish between thin-tailed and fat-tailed kernels. Four of these seven studies lent support to fat-tailed kernels, including plant pathogens as diverse as viruses, fungi and oomycetes. Aerially dispersed pathogens involving, for example, spore propagules or an insect vector such as aphids escaping from plant canopy into turbulent air layer can result in long-distance flights (Ferrandino 1993). In two of these four studies (Rieux et al. 2014; Bousset et al. 2015), exponential-power kernels were fitted to the data. Evidence for fat-tailed kernels was then derived from the confidence interval on the shape parameter ($\tau_{disp} < 1$). These kernels are rapidly varying. Grosdidier et al. (2018) compared a short-tailed kernel to a power-law kernel, the latter being better supported by the data. These very fat-tailed kernels (with regularly varying properties) were significantly better fits to the data. Similar evidence was provided by Gibson and Austin (1996) at the field scale for an epidemic of citrus tristeza virus. This pioneering study demonstrated that a power-law relationship between infective pressure and distance is superior to an exponential one. The Gibson and Austin (1996) study also introduced to plant disease epidemiology – for the first time – the technique of data augmentation, which here facilitates writing a likelihood function and so in turn Bayesian estimation. In particular, the – unobserved – timings of infection are simply treated as additional unknown parameters to be estimated (van Dyk and Meng 2001). However, the particular values of these parameters are then ignored via marginalisation to obtain posterior distributions of

the parameters of interest. Three studies used spatially anisotropic kernels in which propagules can disperse differently depending on the direction (Soubeyrand et al. 2009; Rieux et al. 2014; Grosdidier et al. 2018). These kernels can account for the impact of local wind conditions on aerially dispersed pathogens, and so in principle allow the dispersal tail to be better captured (Savage et al. 2011).

The Effect of Dispersal Kernels on Epidemic Dynamics and Reactive Host-Removal-Based Strategies

Model Overview

We used a simple landscape-scale epidemic model to illustrate how dispersal and model structure can affect epidemic dynamics, as well as to show how performance of a simple reactive control can be assessed. As described in the Online Resource, supplementary to this Chapter, code has been deposited in a freely available repository, allowing readers of this chapter to examine the implementation and performance of the models for themselves. Full technical details of the model are given in the Online Resource; we concentrate here only upon elements required to understand the results we present.

Our metapopulation model tracks pathogen spread across a landscape of discrete, square patches, each of which corresponds to a crop field. In each field we model the numbers of hosts in each of a set of epidemiological compartments, based on infection status. Patches are interconnected through a dispersal kernel, thereby parameterising the spatial spread of the pathogen. The notation, parameters and state variables of the model are summarised in Table 4.2 as well as the reference values used to run simulations (unless stated otherwise in the text). The framework is inspired by Papaïx et al. (2014).

The total extent of the landscape is 3.2 km × 3.2 km and it is partitioned into 1024 square fields each of area 1 hectare. We assume two distinct plant species (or varieties) are cultivated, only one of which is a host for the pathogen (Fig. 4.2a). A proportion p_h =0.5 of "host" fields contain the plant species which can be infected by the pathogen of interest; these fields are distributed at random in the landscape. The same fixed landscape is used in all the simulations presented.

In each field, the epidemiological status of plants is represented using HLIR compartments (Fig. 4.2b). Hosts are therefore distinguished into (H)ealthy, (L)atent (i.e. infected, but not yet able to transmit infection), (I)nfectious (i.e. infected, and able to transmit infection to other fields) and (R)emoved. Transitions between states are modelled using a system of ordinary differential equations (ODEs). The dispersal kernel allows us to estimate the net dispersal probability m_{ij} of pathogen propagules between all pairs of fields (i,j) in the landscape, by integrating over all possible source and recipient host plants in both fields. Power-law and exponential-power kernels parameterised by μ_{disp} and τ_{disp} were considered (see section "Mathematical classification of dispersal kernels").

Table 4.2 Notation, model parameters, state variables and reference values

Notation	Description	Ref value	Unit
Landscape description			
n_f	Total number of fields	1024	
l_f	Side length of an individual square field	100	meters
p_h	Proportion of host fields	0.5	unitless
A_i	Area of field i	10^4	meters^2
Epidemic model: state variables			
$H_i(t)$	Density of healthy plant tissue in field i	na	HTD
$L_i(t)$	Density of latent plant tissue in field i	na	HTD
$I_i(t)$	Density of infected plant tissue in field i	na	HTD
$R_i(t)$	Density of removed plant tissue in field i	na	HTD
$P_i(t)$	Plant tissue in any states in field i	na	HTD
Epidemic model: parameter			
m_{ij}	Dispersal rate from field i to field j	Calculated from other parameters	unitless
μ_{disp}	Mean dispersal distance	80	meters
τ_{disp}	Weight of the dispersal kernel tail	Explored	unitless
r_h	Growth rate of healthy tissue	0.2	HTD TU^{-1}
K	Carrying capacity of each field	10^4	HTD
e	Infection efficiency	10^{-4}	unitless
r_p	Infectious propagule production rate	2.5	SD HTD^{-1} TU^{-1}
ω_L	Mean duration of the latent period	7	TU
ω_I	Mean duration of the infectious period	7	TU
T_{end}	Duration of crop-growing	160	TU
Control strategies			
t_{delay}	Time delay before replanting a field	Explored	TU
r_{ctrl}	Radius of field removal	Explored	meters
th_{ctrl}	Infection threshold for disease first detection	0.2	unitless

TU time unit (days), *HTD* host tissue density, *SD* spore density, *Explored* no reference values are provided for the parameters, since the values used depend on the numerical simulation presented

The epidemic is initiated at $t = 0$ by introducing 0.1% of latently infected plants in a single host field located near the centre of the landscape (Fig. 4.2a). For simplicity, the same field is initially infected in all simulations. Following first infection, plant tissue remains uninfectious for a mean latent period of ω_L time units, but then becomes infectious and produces pathogen propagules for a mean infection period of ω_I. These propagules can infect healthy tissue in the same field (within which the pathogen population is supposed to be perfectly mixed) but also healthy tissue in any other fields of the landscape. In all cases infection between fields i and j is weighted by the dispersal rate m_{ij} as described above.

The default version of our model is based on ODEs, and so is deterministic. However, a stochastic version can readily be derived by replacing each possible transition between states in the ODE system with a single event, which occurs

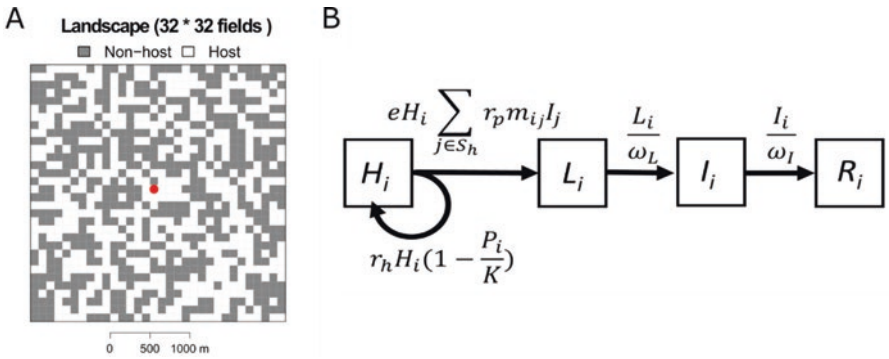

Fig. 4.2 Compartmental model, landscape and dispersal kernel. (**a**) Landscape of 3.2 km × 3.2 km = 10.24 km^2 with 1024 identical square fields of 1 ha each (100 m × 100 m). Host fields are shown in white; non-host fields in grey. In the accompanying code illustrating the ideas of this chapter, the landscape composition (p_h, proportion of host fields) and landscape composition (a_h, level of aggregation of host fields) can be set flexibly and independently, although in all results presented here we fix $p_h = 0.5$ and $a_h = 0$. All epidemics are initiated in the field marked by the red point by introducing 10 latently infected plants at $t = 0$. (**b**) Schematic of the compartmental model used to describe epidemics. In each host field $i \in S_h$, host plants move from healthy (H) to latently infected (L) when first infected; from L to infected (I) after a latent period; and from I to removed (R) once the infectious period is over. P denotes the total density of host tissue. There is also logistic increase of healthy tissue, although in the simulations presented here this only applies following control, since the fields in our model are assumed to start at their carrying capacity of plants

Table 4.3 Transitions and probabilities for the stochastic epidemic model

Description	Transition	Population size n	Rate λ
Healthy birth	$H_i \rightarrow H_i + 1$	$H_i(t)$	$r_h(1 - P_i/K)$
Healthy infection	$\begin{cases} H_i \rightarrow H_i - 1 \\ L_i \rightarrow L_i + 1 \end{cases}$	$H_i(t)$	$e \sum_{j \in S_h} r_P m_{ij} I_j$
End of latency time	$\begin{cases} L_i \rightarrow L_i - 1 \\ I_i \rightarrow I_i + 1 \end{cases}$	$E_i(t)$	$1/\omega_L$
End of infectious time	$\begin{cases} I_i \rightarrow I_i - 1 \\ R_i \rightarrow R_i + 1 \end{cases}$	$I_i(t)$	$1/\omega_I$

stochastically at a rate controlled by the corresponding term in the differential equation (Table 4.3 and the Online Resource). For computational ease, binomial/Poisson draws are used to approximate the number of transitions between compartments occurring during small time intervals. We therefore use a discrete-time approximation to the underlying continuous-time model in the stochastic formulation. Examining the behaviour of this stochastic version of the model relative to the deterministic formulation allows us to systematically understand whether – and how – using a deterministic *vs.* stochastic model affects pathogen dynamics and control.

The total number of transitions of each type (with rate λ) occurring during a small time interval $(t, t + \tau)$ is drawn (i) from a binomial distribution of population size n and probability $p = 1 - \exp(-\lambda\tau)$ for host plants leaving a given compartment to enter another one (events healthy infection, end of latency time, end of infectious time) and (ii) from a Poisson distribution of intensity $n\lambda$ for individuals entering in a given compartment from the "outside" (event healthy birth)

The Effect of the Tail of Dispersal Kernels on Epidemiological Dynamics in the Absence of Control

We first illustrate the effect of the tail of the dispersal kernel on epidemiological dynamics (Fig. 4.3 and Online Resource Fig. OR4.1), initially restricting our attention to the deterministic model. Epidemic dynamics are simulated for the three exponential-power kernels already plotted in Fig. 4.1a. The kernels share the same mean dispers**a**l distance (80 m) but are characterised by increasing tail weight ($\tau_{disp} = \{0.5, 1, 2\}$).

The Gaussian and the exponential kernels both have thin tails, meaning that most disease spread occurs close to the epidemic front. An epidemic travelling wave with constant speed is then observed (van den Bosch et al. 1988) (Fig. 4.3a, b; Supplementary Fig. OR4.1A,B; and the video Supplementary Fig. OR4.5). When $\tau_{disp}<1$ the exponential power-kernel is not exponentially bounded. Long-distance dispersal events are therefore more frequent and induce an accelerating epidemic wave (Kot et al. 1996; Brown and Bolker 2004; Garnier 2011) (Fig. 4.3c; Supplementary Fig. OR4.1C). This type of behaviour, that has been observed for some plant diseases including wheat stem rust (caused by *Puccinia graminis* f. sp. *tritici*), southern corn leaf blight (caused by *Cochliobolus heterostrophus*) and late blight on potato (caused by *Phytophthora infestans*) (Mundt et al. 2009), is most obvious in a video of model simulations (Supplementary Video 4.S1). Note that the constant speed or the accelerating epidemic waves are observed only after initial transitory dynamics within which the infection builds up locally before spreading outwards.

In the absence of control, deterministic and stochastic formulations of the model display rather similar behaviour. This is mostly because with 10 latently infected plants initially introduced (0.1% of the carrying capacity K of the focal field), the probability of initial disease extinction is effectively zero. It should however be emphasised that in general the mean dynamics of the stochastic model do not exactly correspond to those obtained using a deterministic model (Allen and Allen 2003). The major interest of the stochastic model is to allow the underlying variability of an epidemic due to demographic stochasticity to be handled (i.e. to the random variation in the number of new infection events caused by their discrete nature), as we describe below.

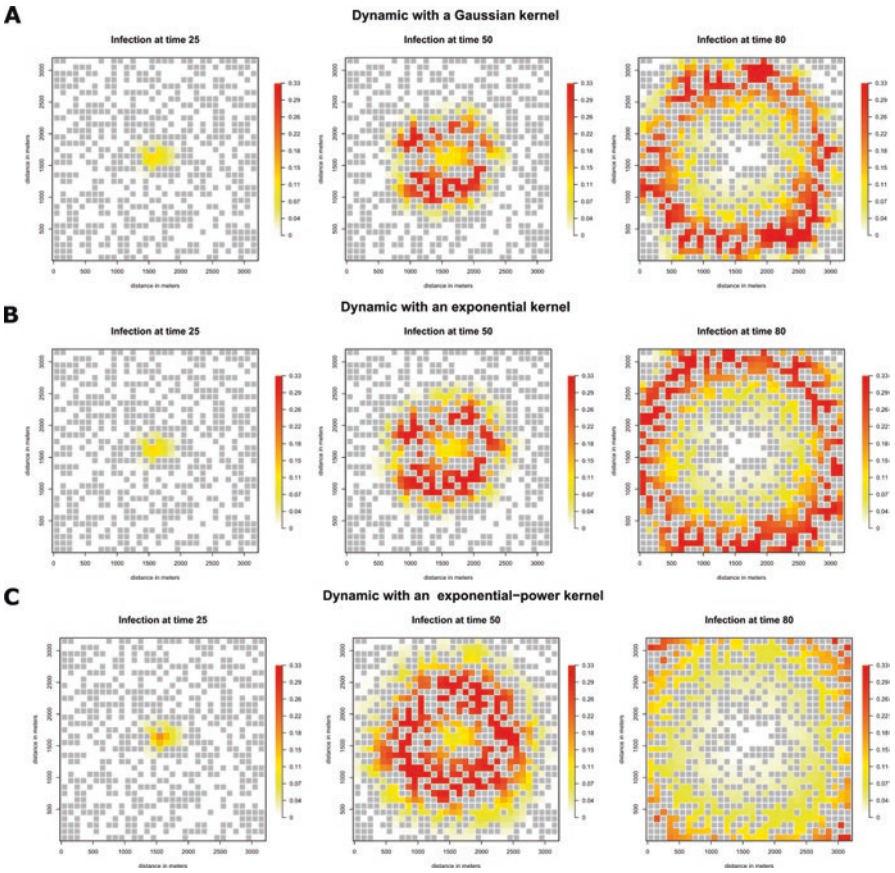

Fig. 4.3 Epidemic dynamics obtained with the deterministic model for three dispersal kernels sharing the same mean dispersal distance (80 m) and having increasing tail weight (Gaussian, exponential and exponential-power with τ_{disp} =0.5). Epidemics are initiated in a single field near the landscape centre. Square symbols indicate non-host fields. **Line A**: Epidemic dynamics obtained with a thin-tailed Gaussian kernel (τ_{disp} =2). The proportion of infectious tissue is displayed at times 25, 50 and 80. The spreading annulus of infectious tissue surrounds a central core region in which plant tissues belong increasingly to the removed compartment (Supplementary Fig. OR4.1). **Line B**: Same as line A for an exponential kernel (τ_{disp} =1). **Line C**: Same as line A for a fat-tailed exponential-power kernel (τ_{disp} =0.5). The three kernels used are illustrated in Fig. 4.1a

Epidemiological Dynamics with Host Removal: Is There an Optimal Radius?

We now focus on a disease management strategy which consists of removing plants from fields contained within a given radius around the detected epidemic focus. In particular we consider a very basic control strategy in which control is only performed a single time. Although this is extremely simple, it is the building block of

the type of repeated reactive disease management that often drives both theory (e.g. Parnell et al. 2009; Cunniffe et al. 2015b; Hyatt-Twynam et al. 2017; Craig et al. 2018) and practice (Gottwald et al. 2002; Peterson et al. 2015; Martelli 2016; Callaway 2016). We specifically investigate how the optimal radius depends on: (i) the characteristics of the dispersal kernels of the pathogen considered and (ii) the mathematical details of the underpinning model.

We assume that disease is detectable in any field within which the sum of the proportions of infected and removed hosts exceeds 0.2. Disease control potentially occurs once per unit of time in our model (at $t = 1,2,3,...$). At the first such time at which disease is detectable in any field across the landscape, all hosts in all fields which have centres lying within a radius r_{ctrl} of any field in which disease is detectable are immediately removed. Removal is therefore initiated around one or more foci of control depending upon whether only one or multiple fields have levels of disease exceeding the detection threshold at the precise time at which control is done. As stated above, disease control occurs only once in our simplified model, and does not occur again thereafter.

We explored values of r_{ctrl} ranging from 50 m, at which radius only the field(s) in which the disease was initially detected are removed, to 2000 m, at which radius the hosts in at least 98% of all fields across the landscape are removed. Removed fields were then either replanted one unit of time after removal ($t_{\text{delay}} = 1$) or were never replanted ($t_{\text{delay}} \to \infty$). This allows us to test whether and how replenishment of susceptible hosts affects the performance of disease management. We quantify performance of disease management at each control radius via the time-integrated amount of healthy tissue across the entire landscape until some notional end time T_{end} as a proxy for crop yield (we present this value normalised relative to the same quantity when there is no control).

We consider first the "modeller's choice" of whether the model is deterministic or stochastic. This turns out to be of fundamental importance (Fig. 4.4). Using the deterministic model, the disease can never be eradicated after it has been introduced. Infection spreads right across the landscape immediately from the very beginning of the epidemic, causing all fields to instantaneously contain at least some pathogen-infected host tissue, albeit sometimes at very low density. This phenomenon is called the "hair-trigger effect" in mathematics (Aronson and Weinberger 1978), and has been referred to as the "Atto-fox" problem in epidemiology (Mollison 1991). Consequently, whatever control radius is applied, the disease can never be eradicated. Depending on whether or not fields are replanted with healthy hosts after removal, the relative performance of control under the yield-based metric we use here therefore either increases monotonically with r_{ctrl} (for $t_{\text{delay}} = 1$) (Fig. 4.4b) or decreases monotonically with r_{ctrl} (for $t_{\text{delay}} \to \infty$) (Fig. 4.4c). The hair-trigger effect is illustrated in Supplementary Fig. OR4.2. Disease is detected at time 27 and fields removed out to a radius of 800 m. At this time, a small proportion of plants is infected outside the control circle, and this allows the pathogen to continue spreading. It follows that no optimal control radius can easily be defined with the deterministic formulation of the simplified model of control we consider here. Nevertheless we note that – for particular disease-spread parameters – there might

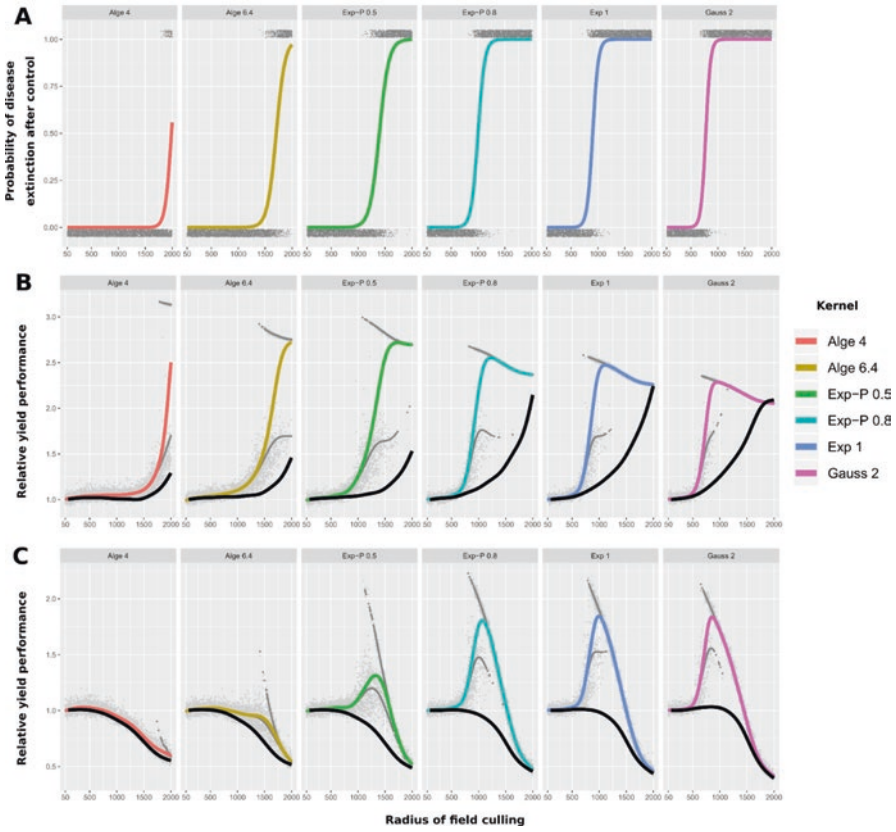

Fig. 4.4 Effects of control radius (r_{ctrl}), dispersal kernel and time delay before replanting (t_{delay}) on the probability of disease extinction after control and on the performance of control strategies. The dispersal kernels used are those plotted in Fig. 4.1a. **Line A**: Probability of disease extinction after control (i.e. control success) as a function of the radius of field removal (r_{ctrl}) for the six kernels considered. **Line B**: Relative performance of each control strategy $Y^{rel}(r_{ctrl}| t_{delay}, ker)$ as a function of the radius of field removal for the six kernels considered with a time delay before replanting of 1 time unit ($t_{delay} = 1$). For each panel, each light grey dot corresponds to performance obtained for each individual run of the stochastic model and darker grey dots to gamma generalise additive model fitted conditionally on whether or not disease extinction occurred after control (see Supplementary Text). The thick black line is the performance obtained using the deterministic model. **Line C**: Same as line B, but in the case for which there is no replanting ($t_{delay} \rightarrow \infty$)

be individual values of t_{delay} which allow an optimum radius to be recovered over the particular timescale of interest.

However, in the stochastic formulation of the model, epidemic eradication becomes possible (Fig. 4.4; Supplementary Fig. OR4.3). In this formulation, the epidemic spreads via discrete probabilistic disease transmission events between infected and healthy individuals. It therefore follows that the epidemic will be eradicated if no infection events have dispersed the pathogen beyond the control radius

before the time of control. For a given control radius and set of disease-spread parameters, eradication is rarely guaranteed, however, although the *probability* of disease extinction increases with r_{ctrl} (Supplementary Fig. OR4.4). Obviously, when this probability is low (typical with power-law kernels), the stochastic and the deterministic models tend to provide fairly similar results.

With the exponential-power kernel family, including members with fat-tailed kernels, extinction becomes certain beyond a given radius in our case study (Fig. 4.4a). The relative performance of the control strategy firstly increases with r_{ctrl} and then decreases beyond a particular radius (Fig. 4.4b, c), allowing an optimal radius of control to be defined. This is because there is a trade-off between the increased probability of disease eradication as the control radius is increased *vs.* the decreased yield that follows over-aggressively removing healthy fields. With the set of parameters used here, an optimal radius always exists irrespective of whether culled fields are replanted just after the control (Fig. 4.4b) or are not replanted (Fig. 4.4c). In the latter case, the highly peaked curves of yield response imply that the precise choice of the control radius is extremely important to maximise control efficiency.

The picture is very different when the two power-law kernels we considered, which we again note are very fat-tailed kernels. One percent of the dispersal events go beyond 639 m using the first power-law kernel ($\tau_{disp} = 4$) and beyond 404 m with the second kernel ($\tau_{disp} = 6.4$). The latter kernel was chosen as it shares the same quantile 99% as the exponential-power kernel with $\tau_{disp} = 0.5$. These two kernels display very similar shapes (Fig. 4.1a). However, despite these apparent similarities, using a power-law rather than exponential-power kernel has a substantial effect on the existence of an optimal control radius, at least as we have defined optimal radius here. With the power-law kernel the probabilities of disease extinction after control are nearly zero for all radii up to 1400 m (which implies removing 61% of the host fields), and even when removing 98% of the host fields ($r_{ctrl} = 2000$ m), the probabilities of disease extinction do not exceed 0.54 ($\tau_{disp} = 4$) and 0.96 ($\tau_{disp} = 6.4$), respectively. Importantly, therefore, even in the stochastic version of our model it is effectively impossible to eradicate the pathogen in a single round of disease control with these fatter-tailed kernels. The consequent difference between the power-law and exponential kernels displaying very similar shapes is particularly striking when fields are not replanted after removal (Fig. 4.4c).

Discussion

In this chapter, we used a simple model to highlight the influence of the interaction between pathogen dispersal kernels and modelling choice in designing optimal control strategies for an emerging epidemic. Since our ambition was simply to draw the reader's attention to a few specific points, we did not perform a full numerical exploration of our model. Nevertheless, the results we present on optimal control radii can potentially guide future research questions, as we outline below.

If initial detection of disease occurs a relatively long time after initial infection – as would be the case, for example, if disease detection was not sufficiently frequent – then the transitory phase within which infection builds up locally would be completed, and so an epidemic wave would already be spreading in the landscape. Larger control radii would then be required to achieve disease eradication, in particular with fat-tailed kernels that result in accelerating waves. However, accelerating waves do not necessarily occur when dispersal kernels are fat-tailed, but instead are conditioned upon the demographic processes involved at low densities (Alfaro and Coville 2017). If population growth is exponential at low density (this is obviously the case with an exponential growth function but is also true with a logistic growth function), an accelerating epidemic wave is always observed with fat-tailed dispersal kernels. However, several mechanisms can prevent this type of low-density exponential growth. For example, strong Allee effects induce growth rates to become negative at low density, as a result for example of reduced fitness due to suboptimal mating opportunities (Hamelin et al. 2016). In this case, no accelerating epidemic waves can be generated from a single source, even with very fat-tailed kernels (e.g. power-law kernels). With weak Allee effects, the population growth rate always remains positive, but can become lower at lower population density. This can occur for example when the probability of infection increases with increasing pathogen dose or if a threshold pathogen dose must be exceeded to overcome host basal immunity (Regoes et al. 2002). In these cases, more subtle interactions between tail fatness and per capita growth rate near zero determine if the spread is accelerating or not. In our view, studies are needed to better characterise how epidemic processes at low densities, in interaction with the form of dispersal kernels, impact reactive control.

Although here we also relied on simulations in a single landscape, using the same pathogen introduction event for each simulation, the code we present can be easily used to study how landscape features impact reactive control. Initial propagation of a newly introduced disease can be strongly impacted by local landscape features (Ostfeld et al. 2005; Plantegenest et al. 2007; Meentemeyer et al. 2012; Papaïx et al. 2014), particularly in interaction with pathogen dispersal. Basic characteristics of a landscape include its composition (i.e. the proportion of different types of habitat, including the fraction of plants that can act as pathogen hosts – defined by p_h in this study) and its configuration (i.e. the specific spatial arrangement of habitat). The framework proposed can easily handle different landscape configurations resulting from varying levels of aggregation of host fields.

The third, fairly severe, restriction on the results presented here is that only a single round of reactive control was considered, i.e. in our simulations, hosts are removed only at one single time. Whenever the disease escapes eradication in this single round of control, the pathogen therefore spreads unperturbed thereafter. As we have seen, and particularly in the deterministic formulation of the model, this has profound effects on the efficacy of disease management. It even means that for our simple situation an optimal radius could not be defined in our stochastic model when there was a very fat-tailed power-law dispersal kernel, because eradication became effectively impossible. However, in practice, disease is controlled more

than once, with multiple rounds of reactive removal. Even if disease is not eradicated, the amount of infection in the system can be greatly reduced by each single round of control. It is therefore possible that repeated controls could "damp down" transmission sufficiently to reduce the (effective) basic reproduction number of the epidemic to smaller than one, thereby controlling the epidemic. This would be concordant with a number of theoretical studies which show that effective reactive control of diseases is possible, even when they spread according to an extremely fat-tailed kernel. Examples of model-based studies showing that control of such epidemics can be successful include models of the animal disease, foot-and-mouth (Tildesley et al. 2006) and a range of plant diseases, including citrus canker (Cunniffe et al. 2015b), huanglongbing (Hyatt-Twynam et al. 2017; Craig et al. 2018) and sudden oak death (Filipe et al. 2012; Cunniffe et al. 2016). These types of studies also tend to highlight the importance of effective disease detection in promoting successful control (Thompson et al. 2016b; Parnell et al. 2015, 2017), since effectively detecting disease means there is a smaller problem to be solved at the time of disease management (Epanchin-Niell et al. 2012).

More generally, we would like to draw the reader's attention to two main points. The first concerns what we have called the "modeller's choice" in deciding to use a deterministic or a stochastic formulation of the model. We have shown that the deterministic model we considered here might not be suitable to generate the types of prediction needed to inform reactive control. Generally speaking, it illustrates that deterministic formulations often become inappropriate at low population densities (Renshaw 1991), via what is sometimes referred to as the "Atto-fox" problem (Mollison 1991; the name comes from unrealistic recovery in infected densities following near eradication in early deterministic models of the spatial spread of rabies in foxes). Predictions concerning the efficacy of spatially explicit reactive control strategies derived via deterministic models therefore require careful interpretation. However, deterministic models can be adapted to allow control to be represented. One obvious way to do so is to relax the formulation of the model to allow epidemic extinction to occur with a deterministic model, as a result of specific interaction between demographic processes and dispersal kernel properties. We note in passing that another choice faced by modellers using deterministic models is whether to use integro-differential equations (IDE), that explicitly represent pathogen dispersal using kernels, or partial differential equations (PDE), that represent pathogen dispersal by a diffusion process. For thin-tailed kernels the spreading dynamics obtained with IDE or PDE approaches do not differ at least qualitatively (Schumacher 1980; Weinberger 1982; Medlock and Kot 2003; Coville et al. 2008). However, the underlying dispersal processes differ and are distinguishable statistically. In particular it is important to emphasise that continuous-time IDE models with Gaussian kernels are in essence different from reaction-diffusion models.

Our second point is that many more studies are needed to inform dispersal kernels of plant pathogens. Several aspects must be taken into consideration. First, in the set of eight studies selected based on the size of experimental design, no studies compared fat-tailed *vs.* very fat-tailed kernels, and more generally none presented results concerning more than two families of dispersal kernels when fitting data.

Doing such a comparison is clearly interesting, particularly since we have seen here that the precise characterisation of the fat-tail weight drastically impacts applied issues such as defining an optimal control radius. However, and this is the second point here, designing experiments to precisely infer dispersal kernels is clearly challenging given the order of magnitude of dispersal of many plant diseases. Accordingly, we only reviewed studies using data gathered in experimental designs of 1 km^2 or greater. It is well known that data confined to relatively small spatial scales can blur the precise estimates of the form of dispersal at large distances, and in particular the shape of the kernel's tail (Ferrandino 1996). Indeed, the spatial scale at which observations are realised is a major concern when fitting and comparing dispersal kernels. Kuparinen et al. (2007) showed how kernels with very different tails may yield similar fits. They found that the predicted dispersal at long distances depends on both the kernel considered and the distances over which the dispersal data was collected. Moreover, observing a single realisation of a dispersal process is not enough to inform the dispersal process. Rather, dispersal outcomes have to be observed under varying spatio-temporal conditions (Kuparinen et al. 2007; Nathan et al. 2012). This is firstly because the basic dispersal process varies according to both biotic factors (genetic effects, plant canopy structure, vector behaviour, etc.) and abiotic factors (landscape features, weather conditions, etc.). In this regard, the difference observed in the dispersal kernels of *Leptosphaeria maculans* for two consecutive transitions from one season to the next – probably due to differences in both climatic conditions and the forces of the inoculum sources – is striking (Bousset et al. 2015). This is also because properly estimating the uncertainty associated with parameters of kernels is an important task.

A first direction to facilitate estimation of pathogen dispersal is to use new sources of host and disease spread data. Integrating new sources of data at different scales will help to better resolve host and pathogen locations. Promising sources of data include unmanned aerial vehicles, remote sensing, and earth observation. There have been recent high-profile successes in detection of a single pathogen from aerial imagery (Zarco-Tejada et al. 2018) . However, accurately distinguishing symptoms caused by different pathogens – as well as distinguishing disease from a more general signature of "stress" – is expected to remain rather challenging (Mahlein 2016).

There are also exciting possibilities that follow from better integrating genomic data into epidemiology. Methods for parameterising pathogen dispersal use data augmentation to integrate over all chains of transmission consistent with observed spread data (Gibson and Austin 1996). Studies of human (Jombart et al. 2014) and animal (Ypma et al. 2012) pathogens show how genomic data can be used to constrain chains of transmission more tightly and so improve the precision of model fits. However, for plant disease models, integration with genetic information is in its very early stages (Picard et al. 2017).

A second direction to improve estimation of pathogen dispersal is to describe dispersal pathways in models in a more realistic way (Cunniffe et al. 2015a). More complex dispersal kernels could be included in forward simulations relatively easily, and are already available for some pathways. These include atmospheric dispersion models (Singh et al. 2011; Meyer et al. 2017) and spread via trade networks

(Shaw and Pautasso 2014). However, attention must be paid to understanding whether including these pathways leads to more accurate prediction, since it is possible that underpinning models will become rather complex.

Recently, Leyronas et al. (2018) provide evidence that the arrival in a given area of airborne inoculum of *Sclerotinia sclerotiorum* from remote origins can be predicted using connectivity networks of air mass movements in the troposphere. In their approach, the directional connectivity between a particular pair of source and sink sites is estimated using archived meteorological data provided by the Global Data Assimilation System (GDAS) of NOAA and the software HYSPLIT that models air-mass trajectories. They also provide evidence that directional connectivity is more informative than the simple geographic distance. This study – as well as other similar studies based on long-distance dispersal of the wheat stem rust pathogen (Meyer et al. 2017) – opens new avenues to understand the atmospheric highways of airborne pathogen dispersal and, from an applied perspective, new opportunities to set up surveillance networks (Carvajal-Yepes et al. 2019).

References

Adrakey, H. K., Streftaris, G., Cunniffe, N. J., Gottwald, T. R., Gilligan, C. A., & Gibson, G. J. (2017). Evidence-based controls for epidemics using spatio-temporal stochastic models in a Bayesian framework. *Journal of The Royal Society Interface, 14*, 20170386.

Alfaro, M., & Coville, J. (2017). Propagation phenomena in monostable integro-differential equations: Acceleration or not? *Journal of Differential Equations, 263*, 5727–5758.

Allen, L. J. S., & Allen, E. J. (2003). A comparison of three different stochastic population models with regard to persistence time. *Theoretical Population Biology, 64*, 439–449.

Almeida, R. P. P. (2018). Emerging plant disease epidemics: Biological research is key but not enough. *PLoS Biology, 16*, e2007020.

Aronson, D. G., & Weinberger, H. F. (1978). Multidimensional nonlinear diffusion arising in population genetics. *Advances in Mathematics, 30*, 33–76.

Bebber, D. P. (2015). Range-expanding pests and pathogens in a warming world. *Annual Review of Phytopathology, 53*, 335–356.

Bousset, L., Jumel, S., Garreta, V., Picault, H., & Soubeyrand, S. (2015). Transmission of Leptosphaeria maculans from a cropping season to the following one. *Annals of Applied Biology, 166*, 530–543.

Boyd, I. L., Freer-Smith, P. H., Gilligan, C. A., & Godfray, H. C. J. (2013). The consequence of tree pests and diseases for ecosystem services. *Science, 342*, 1235773.

Brasier, C. M. (2008). The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology, 57*, 792–808.

Brasier, C. M., & Webber, J. (2010). Sudden larch death. *Nature, 466*, 824–825.

Brown, J. K. M. (1995). Pathogens' responses to the management of disease resistance genes. *Advances in Plant Pathology, 11*, 75–102.

Brown, D. H., & Bolker, B. M. (2004). The effects of disease dispersal and host clustering on the epidemic threshold in plants. *Bulletin of Mathematical Biology, 66*, 341–371.

Brown, J. K. M., & Hovmøller, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science, 297*, 537–541.

Bussell, E. H., Dangerfield, C. E., Gilligan, C. A., & Cunniffe, N. J. (2019). Applying optimal control theory to complex epidemiological models to inform real-world disease management. *Philosophical Transactions of the Royal Society B, 374*, 20180284.

Callaway, E. (2016). Devastating wheat fungus appears in Asia for first time. *Nature News, 532*, 421.

Carvajal-Yepes, M., Cardwell, K., Nelson, A., Garrett, K. A., Giovani, B., Saunders, D. G. O., Kamoun, S., Legg, J. P., Verdier, V., Lessel, J., et al. (2019). A global surveillance system for crop diseases. *Science, 364*, 1237–1239.

Coville, J., Dávila, J., & Martínez, S. (2008). Nonlocal anisotropic dispersal with monostable nonlinearity. *Journal of Differential Equations, 12*, 3080–3118.

Craig, A. P., Cunniffe, N. J., Parry, M., Laranjeira, F. F., & Gilligan, C. A. (2018). Grower and regulator conflict in management of the citrus disease Huanglongbing in Brazil: A modelling study. *Journal of Applied Ecology, 55*, 1956–1965.

Cunniffe, N. J., Laranjeira, F. F., Neri, F. M., DeSimone, R. E., & Gilligan, C. A. (2014). Cost-effective control of plant disease when epidemiological knowledge is incomplete: Modelling Bahia bark scaling of citrus. *PLoS Computational Biology, 10*, e1003753.

Cunniffe, N. J., Koskella, B., Metcalf, C. J., Parnell, S., Gottwald, T. R., & Gilligan, C. A. (2015a). Thirteen challenges in modelling plant diseases. *Epidemics, 10*, 6–10.

Cunniffe, N. J., Stutt, R. O. J. H., DeSimone, R. E., Gottwald, T. R., & Gilligan, C. A. (2015b). Optimising and communicating options for the control of invasive plant disease when there is epidemiological uncertainty. *PLoS Computational Biology, 11*, e1004211.

Cunniffe, N. J., Cobb, R. C., Meentemeyer, R. K., Rizzo, D. M., & Gilligan, C. A. (2016). Modeling when, where, and how to manage a forest epidemic, motivated by sudden oak death in California. *Proceedings of the National Academy of Sciences of the United States of America, 113*, 5640–5645.

Epanchin-Niell, R. S., Haight, R. G., Berec, L., Kean, J. M., & Liebhold, A. M. (2012). Optimal surveillance and eradication of invasive species in heterogeneous landscapes. *Ecology Letters, 15*, 803–812.

Ferrandino, F. J. (1993). Dispersive epidemic waves: I. Focus expansion within a linear planting. *Phytopathology, 83*, 795.

Ferrandino, F. J. (1996). Length scale of disease spread: Fact or artifact of experimental geometry? *Phytopathology, 86*, 685–691.

Filipe, J. A. N., Cobb, R. C., Meentemeyer, R. K., Lee, C. A., Valachovic, Y. S., Cook, A. R., Rizzo, D. M., & Gilligan, C. A. (2012). Landscape epidemiology and control of pathogens with cryptic and long-distance dispersal: Sudden oak death in northern Californian forests. *PLoS Computational Biology, 8*, e1002328.

Freinkel, S. (1997). *American chestnut: The life, death and rebirth of a perfect tree*. Berkeley: University of California Press.

Fry, W. E., & Goodwin, S. B. (1997). Resurgence of the Irish potato famine fungus. *BioScience, 47*, 363–371.

Garnier, J. (2011). Accelerating solutions in integro-differential equations. *SIAM Journal on Mathematical Analysis, 43*, 1955–1974.

Gibbs, J. N. (1978). Intercontinental epidemiology of Dutch elm disease. *Annual Review of Phytopathology, 16*, 287–307.

Gibson, G. J., & Austin, E. J. (1996). Fitting and testing spatio-temporal stochastic models with application in plant epidemiology. *Plant Pathology, 45*, 172–184.

Gilligan, C. A. (2008). Sustainable agriculture and plant diseases: An epidemiological perspective. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*, 741–759.

Gottwald, T. R. (2010). Current epidemiological understanding of citrus huanglongbing. *Annual Review of Phytopathology, 48*, 119–139.

Gottwald, T. R., Sun, X., Riley, T., Graham, J. H., Ferrandino, F., & Taylor, E. L. (2002). Geo-referenced spatiotemporal analysis of the urban citrus canker epidemic in Florida. *Phytopathology, 92*, 361–377.

Grosdidier, M., Ioos, R., Husson, C., Cael, O., Scordia, T., & Marçais, B. (2018). Tracking the invasion: Dispersal of Hymenoscyphus fraxineus airborne inoculum at different scales. *FEMS Microbiology Ecology, 94*, fiy049.

Hamelin, F., Castella, F., Doli, V., Marcais, B., Ravinge, V., & Lewis, M. A. (2016). Mate finding, sexual spore production and the spread of fungal plant parasites. *Bulletin of Mathematical Biology, 78*, 695–712.

Hyatt-Twynam, S. R., Parnell, S., Stutt, R. O. J. H., Gottwald, T. R., Gilligan, C. A., & Cunniffe, N. J. (2017). Risk-based management of invading plant disease. *New Phytologist, 214*, 1317–1329.

Irey, M., Gottwald, T. R., Graham, J. H., Riley, T. D., & Carlton, G. (2006). Post-hurricane analysis of citrus canker spread and progress towards the development of a predictive model to estimate disease spread due to catastrophic weather events. *Plant Health Progress, 7*, 16.

Jombart, T., Cori, A., Didelot, X., Cauchemez, S., Fraser, C., & Ferguson, N. (2014). Bayesian reconstruction of disease outbreaks by combining epidemiologic and genomic data. *PLOS Computational Biology, 10*, e1003457.

Klein, E. K., Lavigne, C., & Gouyon, P.-H. (2006). Mixing of propagules from discrete sources at long distance: comparing a dispersal tail to an exponential. *BMC Ecology, 6*, 3.

Kot, M., Lewis, M. A., & van den Driessche, P. (1996). Dispersal data and the spread of invading organisms. *Ecology, 77*, 2027–2042.

Kuparinen, A., Snäll, T., Vänskä, S., & O'Hara, R. B. (2007). The role of model selection in describing stochastic ecological processes. *Oikos, 116*, 966–974.

Lavigne, C., Klein, E. K., Mari, J.-F., Ber, F. L., Adamczyk, K., Monod, H., & Angevin, F. (2008). How do genetically modified (GM) crops contribute to background levels of GM pollen in an agricultural landscape? *Journal of Applied Ecology, 45*, 1104–1113.

Legg, J. P., Jeremiah, S. C., Obiero, H. M., Maruthi, M. N., Ndyetabula, I., Okao-Okuja, G., Bouwmeester, H., Bigirimana, S., Tata-Hangy, W., Gashaka, G., et al. (2011). Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Research, 159*, 161–170.

Leyronas, C., Morris, C. E., Choufany, M., & Soubeyrand, S. (2018). Assessing the aerial interconnectivity of distant reservoirs of Sclerotinia sclerotiorum. *Frontiers in Microbiology, 9*, 2257.

Mahlein, A.-K. (2016). Present and future trends in plant disease detection. *Plant Disease, 100*, 1–11.

Mahuku, G., Lockhart, B. E., Wanjala, B., Jones, M. W., Kimunye, J. N., Stewart, L. R., Cassone, B. J., Sevgan, S., Nyasani, J. O., Kusia, E., et al. (2015). Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. *Phytopathology, 105*, 956–965.

Martelli, G. P. (2016). The current status of the quick decline syndrome of olive in southern Italy. *Phytoparasitica, 44*, 1–10.

Martinetti, D., & Soubeyrand, S. (2019). Identifying lookouts for epidemio-surveillance: Application to the emergence of *Xylella fastidiosa* in France. *Phytopathology, 109*, 265–276.

Medlock, J., & Kot, M. (2003). Spreading disease: Integro-differential equations old and new. *Mathematical Biosciences, 184*, 201–222.

Meentemeyer, R. K., Haas, S. E., & Václavík, T. (2012). Landscape epidemiology of emerging infectious diseases in natural and human-altered ecosystems. *Annual Review of Phytopathology, 50*, 379–402.

Meyer, M., Cox, J. A., Hitchings, M. D. T., Burgin, L., Hort, M. C., Hodson, D. P., & Gilligan, C. A. (2017). Quantifying airborne dispersal routes of pathogens over continents to safeguard global wheat supply. *Nature Plants, 3*, 780–786.

Mollison, D. (1977). Spatial contact models for ecological and epidemic spread. *Journal of the Royal Statistical Society. Series B, 39*, 283–326.

Mollison, D. (1991). Dependence of epidemic and population velocities on basic parameters. *Mathematical Biosciences, 107*, 255–287.

Morris, C. E., Monteil, C. L., & Berge, O. (2013). The life history of pseudomonas syringae: Linking agriculture to earth system processes. *Annual Review of Phytopathology, 51*, 85–104.

Mundt, C. C., Sackett, K. E., Wallace, L. D., Cowger, C., & Dudley, J. P. (2009). Long-distance dispersal and accelerating waves of disease: Empirical relationships. *The American Naturalist, 173*, 456–466.

Nathan, R., Klein, E., Robledo-Arnuncio, J. J., & Revilla, E. (2012). *Dispersal kernels: Review*. Oxford: Oxford University Press.

Neri, F. M., Cook, A. R., Gibson, G. J., Gottwald, T. R., & Gilligan, C. A. (2014). Bayesian analysis for inference of an emerging epidemic: Citrus canker in urban landscapes. *PLoS Computational Biology, 10*, e1003587.

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144*, 31–43.

Ordonez, N., Seidl, M. F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B. P. H. J., Ploetz, R. C., & Kema, G. H. J. (2015). Worse comes to worst: Bananas and Panama disease – When plant and pathogen clones meet. *PLoS Pathogens, 11*, 1–7.

Ostfeld, R. S., Glass, G. E., & Keesing, F. (2005). Spatial epidemiology: An emerging (or re-emerging) discipline. *Trends in Ecology & Evolution, 20*, 328–336.

Papaïx, J., Touzeau, S., Monod, H., & Lannou, C. (2014). Can epidemic control be achieved by altering landscape connectivity in agricultural systems? *Ecological Modelling, 284*, 35–47.

Parnell, S., Gottwald, T. R., van den Bosch, F., & Gilligan, C. A. (2009). Optimal strategies for the eradication of Asiatic citrus canker in heterogeneous host landscapes. *Phytopathology, 99*, 1370–1376.

Parnell, S., Gottwald, T. R., Gilligan, C. A., Cunniffe, N. J., & van den Bosch, F. (2010). The effect of landscape pattern on the optimal eradication zone of an invading epidemic. *Phytopathology, 100*, 638–644.

Parnell, S., Gottwald, T. R., Cunniffe, N. J., Alonso Chavez, V., & van Den Bosch, F. (2015). Early detection surveillance for an emerging plant pathogen: A rule of thumb to predict prevalence at first discovery. *Proceedings of the Royal Society B: Biological Sciences, 282*, 20151478.

Parnell, S., van den Bosch, F., Gottwald, T., & Gilligan, C. A. (2017). Surveillance to inform control of emerging plant diseases: An epidemiological perspective. *Annual Review of Phytopathology, 55*, 591–610.

Parry, M. F., Gibson, G. J., Parnell, S., Gottwald, T. R., Irey, M. S., Gast, T., & Gilligan, C. A. (2014). Bayesian inference for an emerging arboreal epidemic in the presence of control. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 6258–6262.

Peterson, E. K., Hansen, E. M., & Kanaskie, A. (2015). Temporal epidemiology of sudden oak death in Oregon. *Phytopathology, 105*, 937–946.

Picard, C., Dallot, S., Brunker, K., Berthier, K., Roumagnac, P., Soubeyrand, S., & Jacquot, E. (2017). Exploiting genetic information to trace plant virus dispersal in landscapes. *Annual Review of Phytopathology, 55*, 1–22.

Pimentel, D., Zuniga, R., & Morrison, D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics, 52*, 273–288.

Plantegenest, M., Le May, C., & Fabre, F. (2007). Landscape epidemiology of plant diseases. *Journal of the Royal Society Interface, 4*, 963–972.

Pleydell, D. R. J., Soubeyrand, S., Dallot, S., Labonne, G., Chadœuf, J., Jacquot, E., & Thébaud, G. (2018). Estimation of the dispersal distances of an aphid-borne virus in a patchy landscape. *PLOS Computational Biology, 14*, e1006085.

Regoes, R. R., Ebert, D., & Bonhoeffer, S. (2002). Dose–dependent infection rates of parasites produce the Allee effect in epidemiology. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*, 271–279.

Renshaw, E. (1991). *Modelling biological populations in space and time* (Cambridge studies in mathematical biology). Cambridge: Cambridge University Press.

Rieux, A., Soubeyrand, S., Bonnot, F., Klein, E. K., Ngando, J. E., Mehl, A., Ravigne, V., Carlier, J., & de, B. L. d. L. (2014). Long-distance wind-dispersal of spores in a fungal plant pathogen: Estimation of anisotropic dispersal kernels from an extensive field experiment. *PloS ONE, 9*, e103225.

Rimbaud, L., Dallot, S., Gottwald, T., Decroocq, V., Jacquot, E., Soubeyrand, S., & Thébaud, G. (2015). Sharka epidemiology and worldwide management strategies: Learning lessons to optimize disease control in perennial plants. *Annual Review of Phytopathology, 53*, 357–378.

Rizzo, D., Garbelotto, M., & Hansen, E. (2005). *Phytophthora ramorum*: Integrative research and management of an emerging pathogen in California and Oregon forests. *Annual Review of Phytopathology, 43*, 309–335.

Roy, B. A., Alexander, H. M., Davidson, J., Campbell, F. T., Burdon, J. J., Sniezko, R., & Brasier, C. (2014). Increasing forest loss worldwide from invasive pests requires new trade regulations. *Frontiers in Ecology and Environment, 12*, 457–465.

Savage, D., Barbetti, M. J., MacLeod, W. J., Salam, M. U., & Renton, M. (2011). Can mechanistically parameterised, anisotropic dispersal kernels provide a reliable estimate of wind-assisted dispersal? *Ecological Modelling, 222*, 1673–1682.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution, 3*, 430–439.

Schumacher, K. (1980). Travelling-front solutions for integro-differential equations. I. *Journal für die reine und angewandte Mathematik, 316*, 54–70.

Shaw, M. W., & Pautasso, M. (2014). Networks and plant disease management: Concepts and applications. *Annual Review of Phytopathology, 52*, 477–493.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Bhavani, S., Njau, P., Herrera-Foessel, S., Singh, P. K., Singh, S., & Govindan, V. (2011). The emergence of UG99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology, 49*, 465–481.

Soubeyrand, S., Laine, A. L., Hanski, I., & Penttinen, A. (2009). Spatiotemporal structure of host-pathogen interactions in a metapopulation. *American Naturalist, 174*, 308–320.

Stenlid, J., Oliva, J., Boberg, J. B., & Hopkins, A. J. M. (2011). Emerging diseases in European forest ecosystems and responses in society. *Forests, 2*, 486–504.

Strange, R. N., & Scott, P. R. (2005). Plant disease: A threat to global food security. *Annual Review of Phytopathology, 43*, 83–116.

Talhinhas, P., Batista, D., Diniz, I., Vieira, A., Silva, D. N., Loureiro, A., Tavares, S., Pereira, A. P., Azinheira, H. G., Guerra-Guimaraes, L., Varzea, V., & Silva, M. (2017). The coffee leaf rust pathogen *Hemileia vastatrix*: One and a half centuries around the tropics. *Molecular Plant Pathology, 18*, 1039–1051.

Thompson, R. N., Cobb, R. C., Gilligan, C. A., & Cunniffe, N. J. (2016a). Management of invading pathogens should be informed by epidemiology rather than administrative boundaries. *Ecological Modelling, 324*, 28–32.

Thompson, R. N., Gilligan, C. A., & Cunniffe, N. J. (2016b). Detecting presymptomatic infection is necessary to forecast major epidemics in the earliest stages of infectious disease outbreaks. *PLoS Computational Biology, 12*, e1004836.

Thompson, R. N., Gilligan, C. A., & Cunniffe, N. J. (2018). Control fast or control smart: When should invading pathogens be controlled? *PLoS Computational Biology, 14*, e1006014.

Tildesley, M. J., Savill, N. J., Shaw, D. J., Deardon, R., Brooks, S. P., Woolhouse, M. E. J., Grenfell, B. T., & Keeling, M. J. (2006). Optimal reactive vaccination strategies for a foot-and-mouth outbreak in the UK. *Nature, 440*, 83–86.

Timmerman, V., Borja, I., Hietala, A. M., Kiritis, T., & Solheim, H. (2011). Ash dieback: Pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway. *EPPO Bulletin, 41*, 14–20.

van den Bosch, F., Zadoks, J. C., & Metz, J. A. J. (1988). Focus expansion in plant disease. II: Realistic parameter sparse models. *Phytopathology, 78*, 59–64.

van Dyk, D. A., & Meng, X. L. (2001). The art of data augmentation. *Journal of Computational and Graphical Statistics, 10*, 1–50.

Weinberger, H. F. (1982). Long-time behavior of a class of biological models. *SIAM journal on Mathematical Analysis, 13*, 353–396.

Ypma, R. J. F., Bataille, A. M. A., Stegeman, A., Koch, G., Wallinga, J., & van Ballegooijen, W. M. (2012). Unravelling transmission trees of infectious diseases by combining genetic and epidemiological data. *Proceedings of the Royal Society B: Biological Sciences, 279*, 444–450.

Zarco-Tejada, P. J., Camino, C., Beck, P. S. A., Calderon, R., Hornero, A., Hernández-Clemente, R., Kattenborn, T., Montes-Borrego, M., Susca, L., Morelli, M., et al. (2018). Previsual symptoms of Xylella fastidiosa infection revealed in spectral plant-trait alterations. *Nature Plants, 4*, 432.

Chapter 5
Challenge of Virus Disease Threats to Ensuring Sustained Uptake of Vitamin-A-Rich Sweetpotato in Africa

Jan Kreuze, Wilmer J. Cuellar, and Jan W. Low

Abstract Orange-fleshed sweetpotatoes (OFSP) are a rich source of pro-vitamin A and can alleviate vitamin A deficiency in the developing world. In Africa, traditional varieties have been almost exclusively white-fleshed and introduction and breeding of orange-fleshed varieties into Africa has been severely hampered by virus diseases to which many varieties are susceptible. Breeding progress to generate resistant varieties has been slow due to rare and recessive occurrence of resistance in breeding populations. Production of virus-free seed is complicated by the fact that most sweetpotato viruses show no or only limited symptoms and very low virus concentrations when infected by individual viruses, making them difficult to detect. Even single infections can lead to significant yield losses, but when they combine severe disease complexes are generated, which can lead to total crop failure. Significant efforts have been made in characterizing and understanding virus interactions in sweetpotato over the last two decades to address this challenge; they are reviewed in this chapter. We also review the state of the art in detection of viruses in support of seed systems and breeding. We conclude with recommendations for the most urgent future research directions needed to address virus problems in sweetpotatoes.

J. Kreuze (✉)
International Potato Center (CIP), Lima, Peru
e-mail: j.kreuze@cgiar.org

W. J. Cuellar
International Center for Tropical Agriculture (CIAT), Cali, Colombia
e-mail: w.cuellar@cgiar.org

J. W. Low
International Potato Center (CIP), Nairobi, Kenya
e-mail: j.low@cgiar.org

© The Author(s) 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_5

Justification for Introducing OFSP and the Need for Quality Seed and Virus-Resistant Varieties

For whatever reason, when sweetpotato was introduced to Africa from the Americas over 500 years ago (O'Brien 1972), the types of sweetpotato that came to dominate were white-fleshed, containing no beta-carotene, or yellow-fleshed, which can have limited amounts of beta-carotene. Today in sub-Saharan Africa (SSA), 48% of children under five years of age are estimated to be vitamin A deficient (Black et al. 2013). The main causes of vitamin A deficiency (VAD) are lack of intake of vitamin-A-rich foods and illness, which leads to poor absorption or the loss of vitamin A (Sommer and West 1996). Significant reduction of VAD in young children can lower the incidence of young child mortality by over 20% and improved access to vitamin-A-rich foods and reducing illness are part of a long-term sustainable solution (Stevens et al. 2015). Thus, given the high level of VAD in SSA and the fact that sweetpotato is widely grown throughout SSA as a food security crop, with 4.7 million hectares in production (FAO 2017), the rationale for introducing and promoting orange-fleshed sweetpotato (OFSP) on the continent is obvious. Moreover, sweetpotato is principally considered a woman's crop and a crop of the poor in SSA (Low et al. 2009), so the intervention naturally targets the vulnerable groups most at risk of vitamin A deficiency.

One of the first studies to investigate the acceptability of OFSP varieties in SSA was undertaken among 20 women's groups in Western Kenya in 1995–1996 (Low et al. 1997). The research demonstrated that the orange color of the root was highly acceptable to both children and women, but the challenge was the texture with young children preferring low dry-matter types (like those found in the USA), while adult women demanded high dry-matter types (>30%) with mealy textures similar to the dominant, local white-fleshed types (Hagenimana et al. 1999). However, nearly all OFSP varieties imported from outside of SSA that were introduced to East Africa failed within a few years due to Sweet Potato Virus Disease (SPVD) (Fig. 5.1) (Grüneberg et al. 2015). Initial progress in uptake and utilization of OFSP imported varieties was faster in Southern Africa, where virus pressure was lower and adult dry-matter preferences (26–28%) also lower than those found in East Africa (Low et al. 2017b). In East Africa, however, we were fortunate to locate two orange-fleshed landraces (Kakamega, Ejumula) that could be utilized as varieties and as parents in breeding programs.

In part due to the swathe of bi-modal rainfall areas found in East and Central Africa, over half of sweetpotato production in SSA is concentrated in this zone. Sweetpotato is vegetatively propagated in this region, principally by taking cuttings from existing plants, or in cases where the dry season is longer, leaving a few roots unharvested, which sprout again when the next rains arrive. Traditionally, if one does not have sufficient planting material on one's own field, one can easily receive planting material from a neighbor for free (McEwan et al. 2015). Clearly, this is the ideal environment for viruses to accumulate over time in a given variety. While most farmers do not know what a virus is, they note that the plant "is getting tired" or the plant is "sick". Sales of planting material are mostly to those traveling from outside the immediate community.

Fig. 5.1 Orange-fleshed sweetpotato variety 'Kakamega', showing severe symptoms of SPVD (right), next to a symptomless plant (left). (Photo: Segundo Fuentes)

In this context, during the past 15 years, the International Potato Center (CIP) and 13 national partners in SSA have developed a two-pronged approach for tackling the often devastating effects of SPVD: (1) Breeding for virus resistance, and (2) developing sustainable quality "seed" systems.

The CIP virus resistance breeding effort is concentrated in Uganda and undertaken in collaboration with the National Crops Resources Research Institute (NaCRRI). This effort can be described as searching for a needle in a haystack, given that sweetpotato is a hexaploid (2n = 6× = 90) and the virus resistance level found in breeding populations to date occurs at very low frequencies of ≤0.2%. The two viruses constituting SPVD are Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV), in which inheritance of resistance is supposedly recessive (Mwanga et al. 2017). Moreover, resistance working against one virus strain may not work against another strain of the same virus. In spite of this, several high-yielding, moderately resistant varieties have been released during the past 12 years (Mwanga et al. 2009, 2016), the most notable being Kabode (NASPOT 10 O), which has also been released by Kenya, Tanzania, and Rwanda.

In the future, we expect more rapid progress in virus resistance breeding through the use of breeding schemes exploiting heterosis that will allow breeders in high SPVD pressure zones to apply more inbreeding for SPVD resistance (two partially inbred genepools) without sacrificing heterozygosity (hybrid population) for yield and stability performance (Mwanga et al. 2017). In addition, breakthroughs in ongoing molecular marker research could vastly accelerate progress (Gruneberg et al.

2015). Clearly, low-cost but accurate ways to screen for different viruses at scale would also contribute to more efficient and timely selection decisions.

Regardless of the variety used, timely access to quality seed (actually cuttings from a vine rather than true seed) is essential for high yields in any sweetpotato production system. Virus-free planting material is higher yielding than non-virus-free (Adikini et al. 2016). On women's small landholdings in Rwanda, use of quality seed was associated with increased yield; there were then surplus roots to sell, generating on average $277 annually per household (Sindi et al. 2015). In China, the introduction of virus-free seed to 80% of a major growing area by public-sector extension led to an average yield increase of 30% (Fuglie et al. 1999) .

Our efforts have focused on collaborating with 11 national programs and two private-sector companies to improve the efficiency and sustainability of early-generation seed (EGS) production, which consists of tissue culture plantlet production, and subsequent multiplication in a screenhouse, following a business model (Rajendran et al. 2017). Clearly, ensuring that all pre-basic materials are virus-free is a core part of their mandate. Given the low multiplication rates of sweetpotato compared to grain crops and the perishability and bulkiness of the vines, coupled with limited willingness of growers to pay, companies specializing in grain seed sales have shown little interest in investing in sweetpotato seed (Low et al. 2017a). Hence, in collaboration with non-governmental organizations and government extension services, the focus has been on setting up a network of decentralized, trained vine multipliers (DVMS) to serve their surrounding communities. These DVMS, in turn, are served by a few larger, well-resourced basic multipliers in their districts (or equivalent administrative unit) (McEwan et al. 2015). Multipliers are encouraged to become commercial root producers as well, as demand for seed often fluctuates. Several SSA countries are now implementing more formal certification or quality-declared seed classifications at this stage in the multiplication process to ensure that farmers know what variety they are receiving and their level of quality (McEwan et al. 2012). To make such classification schemes work in the long-run, affordable and accurate diagnostic tools for virus detection are required, so that ensuring quality does not become a bottleneck, impeding farmer access to clean planting material of improved varieties.

Thus, to achieve progress in both breeding for virus resistance and establishing sustainable access to seed, a better knowledge of how viruses operate, and how the ones causing serious economic damage can be combatted, are urgent priorities.

Brief View of Progress Made in Detecting Viruses in Africa

Being a vegetatively propagated crop, sweetpotato is prone to the buildup of pathogens in planting material. Because planting material is largely produced through stem cuttings (even when roots are used as the primary multiplication material) these are largely limited to foliar diseases, and particularly viruses. Sweetpotato is known to be affected by over 30 viruses (Clark et al. 2012), most of which are recently described DNA viruses (Table 5.1). However, studies have consistently

Table 5.1 Viruses reported to infect sweetpotato worldwide

Family	Genus	Virus	Vector
Potyviridae	*Potyvirus*	SPFMV, SPVC, SPVG, SPV2, SPLV, SPMSV, SPVMV, SPYDV	Aphids
	Ipomovirus	SPMMV	Whiteflies?
Closteroviridae	*Crinivirus*	SPCSV	Whiteflies
Betaflexiviridae	*Carlavirus*	SPCFV, SPC6V	
Geminiviridae	*Begomovirus*	>10	Whiteflies
	Mastrevirus	SPSMV	
Caulimoviridae	*Badnavirus*	SPPV	
	Solendovirus	SPVCV	
	Cavemovirus	SPCV	
Luteoviridae	*Polerovirus*	SPLSV	
Bromoviridae	*Cucumovirus*	CMV	Aphids
Secoviridae	*Nepovirus*	SPRSV	

shown that the potyviruses SPFMV and SPCSV are the most widespread and damaging in tropical regions of the world. In Africa, besides SPFMV and SPCSV, the potyvirus Sweet potato virus C (SPVC, previously known as the C strain of SPFMV) is also widespread and often found in association with SPFMV. Other potyviruses such as Sweet potato virus G (SPVG) and Sweet potato virus 2 (SPV2) are also found, as are the ipomovirus Sweetpotato mild mottle virus (SPMMV) (Ateka et al. 2007; Rännäli et al. 2009; Tugume 2010), begomoviruses (Miano et al. 2006; Wasswa et al. 2011), the carlaviruses Sweet potato chlorotic fleck virus (SPCFV) (Aritua et al. 2007) and Sweet potato C6 virus (SPC6V) (De Souza et al. 2013), the cavemovirus Sweet potato collusive virus (SPCV, previously known as Sweet potato caulimo-like virus) (Cuellar et al. 2011b), and the cucumovirus Cucumber mosaic virus (CMV). The vectors of these viruses, where known, are aphids (potyviruses and CMV) and whiteflies (SPCSV and begomoviruses). Except for CMV, all these viruses are unique to sweetpotato, and sweetpotato (or related Ipomoea spp.) is not affected by viruses infecting other crops, nor are sweetpotato viruses found to infect other host plants, suggesting that the plant provides some unique cellular environment in which only specialized viruses can propagate.

Whereas SPFMV by itself usually causes only mild or no symptoms and limited or no yield loss in plants when it is the only virus infecting the plant, in combination with SPCSV it generates the severe sweetpotato virus disease complex (SPVD), which can lead to yield losses of close to 100% when grown from infected planting material (Clark et al. 2012; Gibson and Kreuze 2015). SPCSV can also cause synergistic diseases with other viruses, including SPMMV, SPCFV, CMV, begomoviruses, SPCV and the Solendovirus sweet potato vein clearing virus (SPVCV) (Untiveros et al. 2007; Cuellar et al. 2011a, 2015). This makes SPCSV the most significant virus contributing to sweetpotato yield losses worldwide. However, recent studies have provided evidence that SPFMV and begomoviruses by themselves may also cause significant yield losses in several different varieties (Ling et al. 2010; Mulabisana et al. 2019).

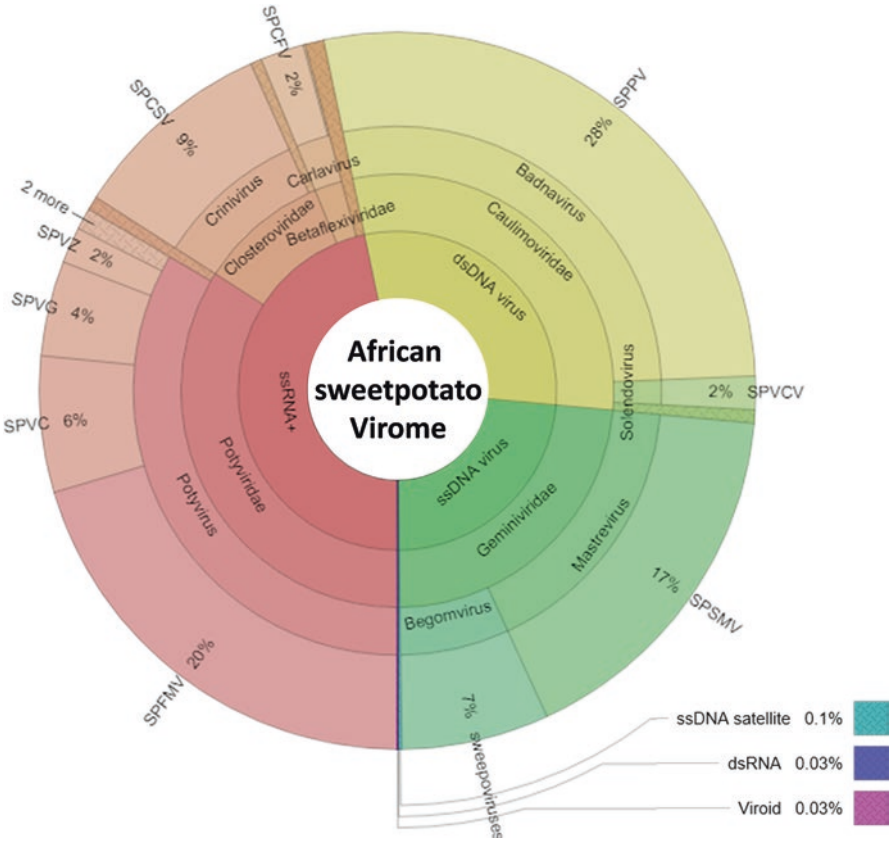

Fig. 5.2 Viruses identified in African sweetpotato samples indicating the percentage of all viruses identified in the study. Viruses are organized according to taxonomic classification

Despite this, except for Uganda and to a lesser extent Kenya, Tanzania and Rwanda, where surveys have been performed, until recently it was unclear how frequently all the different viruses occur and to what extent they contribute to yield losses throughout the African continent. The African sweetpotato virome project (Kreuze and Fei 2019) (http://bioinfo.bti.cornell.edu/virome/) applied high-throughput sequencing and assembly of small RNAs (sRSA) for virus detection to field-collected samples from 11 different African countries across the continent from 2012 to 2014. Results (Fig. 5.2) revealed that the most frequently occurring viruses were the recently discovered badnaviruses, Sweet potato pakakuy virus (SPPV; 76% of samples), and the mastrevirus Sweet potato symptomless mastrevirus, which was also extremely common (45% of samples). Little is known about these viruses, but since their discovery through sRSA a decade ago they have been reported from sweetpotatoes around the world. They are not associated with any symptoms or disease, and our work (Kreuze et al. 2020) indicates that badnaviruses are not genome integrated, and occur in extremely low titers, and cause no appreciable symptoms or interaction with other viruses in sweetpotato or the indicator

plant *Ipomoea setosa*. Thus, they may represent a new class of commensal or endosymbiotic viruses that are likely to exist and be discovered as new ultrasensitive virus detection approaches are applied more widely. Hence, when considering only viruses known to cause disease in sweetpotatoes, SPFMV was found to be the most common virus (55%) followed by SPCSV (25%), begomoviruses (18%) and SPVC (17%). In contrast, SPVG, SPV2 and a new potyvirus identified and named SPVZ appeared to be locally common in some countries but were not widespread over the continent. Several new viruses were also identified, but most represented only a fraction of the total and thus are not likely to be significant.

The begomoviruses were among the most common viruses found throughout the continent, something that was not known previously, yet little is known about their impact on yield. Studies performed previously in the USA showed they could have significant yield impacts, depending on the varieties, but are largely symptomless (Ling et al. 2010). Similar results were recently reported from different varieties in South Africa (Mulabisana et al. 2019). Recent results from yield trials in Kenya show a similar picture with apparently strong yield impacts in one variety, but none in another (Wanjala et al. 2019). Interestingly, the variety most affected by begomovirus was more resistant to SPVD, suggesting there might be a tradeoff for resistance to SPVD and begomoviruses, as previously suggested (Wasswa et al. 2011). Research should be prioritized to elucidate this point, because if it is confirmed, breeding for resistance to SPVD might inadvertently lead to selection of susceptibility to begomoviruses, replacing one problem with another. An additional result from the sweetpotato virome project was that visual symptoms on the plant, as determined by an expert, had little diagnostic value in relation to the viruses found in the plants under African field conditions.

Based on the studies described above, it can be concluded that the most important viruses to consider in Africa are SPFMV, SPVC, SPCSV and probably begomoviruses, although the extent of their yield impact seems highly variable. Nevertheless, other viruses can be locally common and should be considered in these locations when testing for viruses in production of clean planting material. To enable this, specific, sensitive, easy and affordable diagnostic tests need to be developed for these viruses, which are discussed in another section of this chapter.

Understanding Viruses to Improve Breeding for Virus Resistance

Virus-infected plants can gain resistance to further infections by closely related viruses, a phenomenon known as ‘cross-protection’, or they can gain susceptibility to viruses that otherwise would not cause disease in a single infection, a phenomenon known as ‘synergistic interaction’ (Kassanis 1964). The biological study and characterization of mixed virus infections versus host defence responses has revealed important genetic and biochemical phenomena common to all living

organisms. Early studies identified that infection by a virus affects the physiology, metabolic activity or even structure of the plant and any of these induced changes will affect the ease with which the host will respond to a second virus challenge (Matthews and Hull 2002; Smith 1931, 1960). As mentioned above, sweetpotato is vegetatively propagated and mixed virus infections do accumulate over time, representing a growing challenge for the plant. One well characterized host response to virus infection is based on 'RNA silencing', whereby host proteins identify the viral RNA and cut it into small molecules of 21–24 nt. These viral-derived RNA molecules then trigger a sequence-specific host response that degrades the RNA of the invader virus (Baulcombe 2004; Ding and Voinnet 2007). Therefore, it is not unexpected that viruses have evolved to encode proteins that block RNA silencing. Such proteins are known as RNA silencing suppression (RSS) proteins and several of them had been identified in the past as virulence factors or pathogenicity determinants (Díaz-Pendón and Ding 2008). RSS proteins, belonging to different families of viruses, inactivate the RNA silencing response at different points. Some are more effective than others in counteracting this host defence. One such protein, HCpro, is encoded by viruses in the genus *Potyvirus* and has been known for more than 20 years as a pathogenicity determinant and mediator of viral synergisms (Vance et al. 1995; Pruss et al. 2004). At the turn of the century there were around 20 different virus species reported to infect sweetpotato in single or mixed infections, including several potyviruses such as SPFMV (Loebenstein et al. 2003). However, potyviruses in sweetpotato were not mediators of viral synergisms in this host.

The Biggest Challenge: Sweet Potato Virus Disease (SPVD)

Several complex viral diseases of sweetpotato have been characterized and SPCSV has been identified as one of the components of the mixed infection: SPCSV in complex with SPFMV and Sweet potato mild speckling potyvirus (SPMSV) causes a chlorotic dwarf disease reported in Argentina (Di Feo et al. 2000). SPCSV and Sweet potato mild mottle ipomovirus (SPMMV) cause a severe mosaic disease reported in Uganda (Mukasa et al. 2006). In experimental inoculation tests, SPCSV could enhance the severity of disease symptoms caused by several RNA viruses including carlaviruses and cucumoviruses (Untiveros et al. 2007). What is most impressive is that the effect SPCSV has on mixed infections is not limited to co-infections with RNA viruses, but also occurs with DNA viruses such as the caulimoviruses (Cuellar et al. 2011b) and begomoviruses (Cuellar et al. 2015). Molecular analyses of mixed-virus infections involving SPCSV revealed that the disease symptom severity increases as the accumulation of the co-infecting virus(es) rises, while the titres of SPCSV remain little affected (Gibson et al. 1998; Karyeija et al. 2000; Mukasa et al. 2006; Untiveros et al. 2007; Kokkinos and Clark 2006a).

SPFMV and SPCSV are distributed worldwide including the Americas (Gutiérrez et al. 2003; Kashif et al. 2012; Di Feo et al. 2000) and Asia (Milgram et al. 1996;

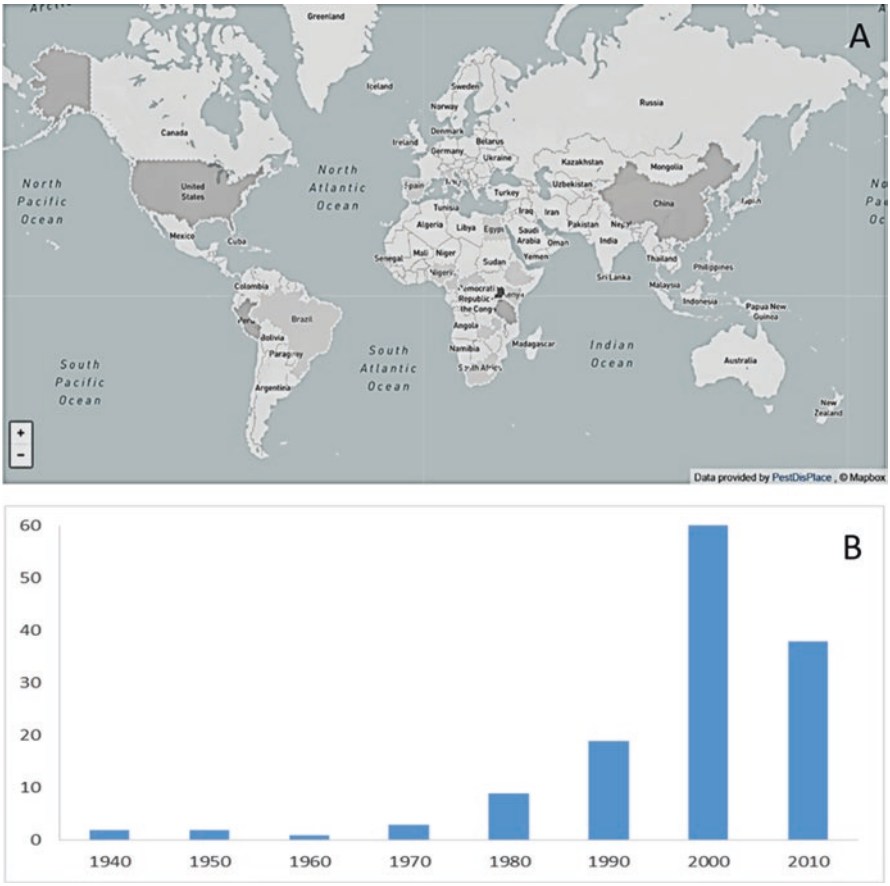

Fig. 5.3 Global distribution of SPVD field reports published between 1940 and 2019. (Source: Google Scholar). (**a**) Country shading indicates number of reports; at every tenth report, the shading of the country increases; Uganda shows the highest number of SPVD reports (>30). (**b**) Cumulative number of SPVD field reports published per decade, between 1940 and 2019. The y axis indicates number of publications during the decade starting from the year indicated on the x axis

Qiao et al. 2011), yet cause the most damage in Africa (Gibson et al. 1998; Ateka et al. 2004; Njeru et al. 2008; Njeru et al. 2004; Mukasa et al. 2003; Mukasa et al. 2006; Tairo et al. 2005; Fenby et al. 2002). In Africa, epidemics of SPVD have been associated with the disappearance of elite cultivars (Gibson et al. 1997). A significant rise in the number of scientific reports on SPVD over the last 20 years (Fig. 5.3), linked to advances in virus detection protocols and the functional characterization of the disease, is a reflection of the relevance that SPVD still has in the developing world. Particular focus has been on two RSS proteins encoded by SPCSV, p22 and p26 (Kreuze et al. 2005).

The Role of RSS Proteins in Virus Susceptibility

Historically p22 protein was the first candidate for an RSS protein involved in SPVD. Several factors contributed to this. First, p22 was present in the first SPCSV isolate from Uganda that was completely sequenced (Kreuze et al. 2002). Because at the time only partial sequencing (mainly regions corresponding to the Coat Protein or the Replicase) was the minimum required to identify other isolates of the virus, the absence of p22 in most non-Ugandan isolates was overlooked (Cuellar et al. 2008). Second, like other RSS proteins, p22 lacks any detectable sequence homology with other proteins and was located in a genomic region where other RSS proteins had been identified in the *Closteroviridae* (the family of viruses to which SPCSV belongs). Third, p22 readily showed RSS activity in standard agro-infiltration tests done in *Nicotiana benthamiana* plants (Kreuze et al. 2005). Efforts to clone and identify the diversity of p22 sequences from different isolates of SPCSV unveiled the fact that p22 was absent in most SPCSV isolates known so far (Cuellar et al. 2008). Interestingly, all those isolates lacking p22 were known to mediate SPVD, therefore any role of p22 in the development of the diseases was discarded. After conducting larger surveys for SPCSV isolates, based either on sequencing of the targeted 3′ region of RNA1 of SPCSV and generic deep-sequencing protocols (Kreuze et al. 2009), it was found that p22 was encoded in most of SPCSV isolates from Uganda but was absent elsewhere (Kashif et al. 2012; Tugume et al. 2013). At the same time, agro-infiltration assays of several SPCSV proteins identified a weak but still detectable RSS activity for the \p26 protein. P26 encodes an RNase III-type of protein (RNase3). Proteins in this family contain a single ribonuclease domain and a single dsRBD domain. Bacterial and viral RNAse III belong to this class (MacRae and Doudna 2007). Unlike other viral silencing suppression proteins, homologs of the viral RNase3 enzymes exist in unrelated RNA and DNA viruses (Weinheimer et al. 2015). All SPCSV isolates characterized so far encode an RNase3; this also occurs in SPCSV-related species as shown by its detection in wild *Ipomoea* plants. In contrast, p22 has been found in few isolates so far (Tugume et al. 2013), which suggests an active genomic region in SPCSV (the 3′ end of RNA1) moulded by recombination that could serve as an important target for engineering resistance to SPVD. Experiments by Cuellar et al. (2009) unequivocally demonstrated that expression of p26 by itself was sufficient to generate synergistic diseases with all other viruses tested, implicating it in the inhibition of a key antiviral defence mechanism in sweetpotato. Whereas the exact molecular mechanism by which RNase3 suppresses the plant RNA silencing response has yet to be determined, we know it requires dsRNA ribonuclease activity (Cuellar et al. 2009; Weinheimer et al. 2015) and that it interacts with the plant-encoded antiviral defence protein SGS3 (Weinheimer et al. 2016). This protein domain is a prime target for biotechnological engineering of resistance and for developing strategies to control the diseases and minimize economic losses in sweetpotato production.

The other major component of SPVD, SPFMV has also been studied for its ability to suppress RNA silencing. SPFMV, together with SPVC, SPVG and SPV2 belong to the genus *Potyvirus* and are related to each other in having a significantly

larger genome than other potyviruses due to an exceptionally large P1 protein. Untiveros et al. (2007) recognized the existence of two domains in the P1 protein, named P1-N and P1-Pro, separated by a hypervariable domain. Then Clark et al. (2012) recognized that the P1-Pro domain had an overlapping open reading frame, which they named PISPO. This was subsequently identified as an RNA silencing suppressor unique to *Ipomoea*-infecting potyviruses (Untiveros et al. 2016; Mingot et al. 2016). The P1 itself and HC-Pro of sweetpotato-infecting potyviruses, however, also show silencing suppressor activities (Rodamilans et al. 2018). This implicates at least three RSS proteins with different modes of action in SPFMV. In addition, the polymerase slippage rate, leading to production of P1N-PISPO, is reduced in SPVD-affected plants, adding to the complexity of the interaction of this disease.

The Role of Wild Ipomoea *Weeds in Virus Epidemiology*

Long before plants were domesticated, viruses co-evolved with their wild host plants (Lovisolo et al. 2003). This co-evolution was drastically affected following plant domestication, agricultural intensification of monocultures and trade (Stukenbrock and McDonald 2008). Several studies suggest that species of wild host plants play the role of reservoirs in the ecology of plant viruses and, therefore, in their epidemiology as sources of inocula and diversity in agroecosystems (Fargette et al. 2006). Over 80 species of the genus *Ipomoea* and other genera within the family Convolvulaceae occur in East Africa. They are important reservoirs of a larger diversity of SPFMV isolates and their corresponding RSS proteins (Tugume et al. 2010a, b, 2013, 2016, 2008). Furthermore, at least one new viral species related to SPCSV and encoding an RNase3-like RNA-silencing suppressor protein has also been detected in Uganda (Tugume et al. 2013) and Tanzania (African Sweetpotato Virome), although this virus seems to be currently rare in cultivated sweetpotato.

Resistance

Whereas a significant amount of research has been done to understand susceptibility to SPVD during the last 20 years, almost no work has been done to understand the mechanisms of resistance. This is probably largely due to the lack of highly resistant genotypes, the complex nature of the highly heterozygous hexaploid sweetpotato genome and lack of major genes contributing to virus resistance in sweetpotato germplasm. With more resistant genotypes now being produced through breeding efforts and genomic tools such as a reference genome for sweetpotato (Wu et al. 2018) available, new opportunities have arisen to address this lack of understanding and doing so should be a priority. Whereas resistance has been described in some related *Ipomoea* spp. (Karyeija et al. 1998), these do not cross with sweetpotatoes and are thus of limited value for breeding purposes.

Understanding Viruses to Improve Diagnostic Tools and to Support Breeding and Development of a Quality Seed System

Generally speaking, sweetpotato is rather resistant to most viruses: only specialized sweetpotato viruses seem to be able to infect it, and even they usually cause only mild or no symptoms and occur in only low titres when infecting sweetpotato alone. Many varieties also seem to be able to recover from single infections, producing virus-free branches from which cuttings can be taken, and this is more pronounced in varieties considered as resistant to SPVD. This phenomenon, which becomes more pronounced at higher temperatures, may explain why farmers in Africa can maintain planting material over many generations without it completely degenerating through virus infections (Gibson and Kreuze 2015). Whereas the mechanism for this recovery is not yet elucidated, it is likely to be related to the efficiency of resistance in the plant mediated by RNA silencing; this has also been shown to be temperature dependent (Szittya et al. 2003). If RNA-silencing-mediated resistance is critical for recovery and/or for virus resistance in the field, as is suggested by several lines of evidence (role of RNase3 in SPVD, temperature dependence of recovery), understanding its mechanism and the genes governing it should enable more efficient breeding for resistance and other control mechanisms.

Small RNAs are key molecules in the RNA silencing pathway and studying them might further contribute to understanding virus resistance and susceptibility in sweetpotato. Indeed, when the sRSA technology was invented and first applied to sweetpotato, an immediate result was the discovery of previously unknown viruses (Kreuze et al. 2009). Whereas sRSA has evolved into a widely adopted method for the discovery and identification of viruses in plants, it also enables the study of RNA-silencing-based antiviral defence in these same plants (Pooggin 2018). Indeed, the original intent of the experiment by Kreuze et al. (2009), was to try to understand how SPFMV and SPCSV were targeted by the plant RNA silencing machinery in order to enable more informed design of transgenic constructs for resistance to SPVD. Previous attempts to generate resistance to SPVD by targeting both viruses with RNA-silencing-inducing constructs had been only partially successful: these led to reduced titres of SPCSV, but were not sufficient to prevent the provocation of SPVD in co-infection with SPFMV (Kreuze et al. 2008). Based on that analysis, new constructs were designed and unpublished results from field trials in Kenya show promising levels of resistance. Likewise, experiments are ongoing in our lab to compare small RNA profiles/amounts, viral RNA concentrations and host gene expression in varieties considered susceptible, moderately resistant and resistant to SPVD. This will certainly lead to new insights that may be applied to guide and support breeding efforts.

The fact that most sweetpotato viruses occur at only low concentrations in plants when infecting alone, presents a diagnostic problem. Whereas the International Potato Center has for many years, produced virus detection kits for the most common sweetpotato viruses based on Enzyme linked immunosorbent assays (ELISAs), the reliability of detection directly from sweetpotato by ELISA is limited in single

virus-infected plants. The only way to reliably detect such viruses is through grafting plants to the universal indicator plant *I. setosa* and performing the ELISA from that plant; this is a laborious and time-consuming procedure, only worthwhile for the most basic nuclear stock of planting material. More sensitive laboratory-based methods such as (multiplex) PCR (Kwak et al. 2014; Li et al. 2012; Opiyo et al. 2010) and qPCR (Kokkinos and Clark 2006b) have been published and are available for testing most sweetpotato viruses, but have the disadvantage of being relatively expensive, requiring laboratory conditions for sample preparation and running the actual test. Thus, like indicator grafting, these are typically only applied to nuclear stock material. However, new sensitive diagnostic tools are under development that could significantly reduce the time and cost of virus testing and could be used at the point of care, for example on seed production fields.

Although sRSA is also relatively expensive, requiring laboratory conditions, skill and bio-informatics capacity, it has a tremendous potential to replace or accelerate virus indexing procedures to produce -virus-free nuclear stock material and to enable quarantine procedures. The current gold standard for virus indexing sweetpotato consists of two rounds of grafting to *I. setosa*, combined with PCR for DNA viruses and NCM ELISA for 10 other viruses. The whole procedure takes at least 6 months, a considerable amount of greenhouse space and skill – at a cost of more than 120 USD per sample. This procedure ensures that all possible viruses are reliably detected, including those variants that are as yet undescribed since they also may produce symptoms in the indicator plant. sRSA can likewise detect all known as well as unknown viruses, but directly from the query plant itself, avoiding the need for the use of an indicator plant. The procedure to prepare samples takes about a week; high-throughput sequencing takes a few days, and analysis a few hours using specialized software, thus providing a significant reduction in time to result, which is currently the biggest bottleneck in moving planting material of improved varieties and breeding materials between countries. Another benefit of sRSA is that data once generated can be saved and if new viruses are discovered in the future, material does not need to be re-tested; one can simply re-query the sequence data for its presence. With the cost being below 100 USD per sample combined with manifold reductions in time to results, sRSA is an obvious candidate to replace the current indicator-host-based indexing procedure. At present, validation data are being generated and standard operating procedures developed for sRSA to replace standard indexing, which is under ISO17025 certification at CIP headquarters in Lima. Once this has been done, sRSA can be fully implemented, replacing indicator host indexing. Training provided by CIP to national programs during recent years aims to ensure they become familiar with sRSA as well, and improvement of user-friendliness of analysis software may eventually lead to this technique being widely adopted throughout the world.

sRSA has already been widely applied to identify new viruses and as a survey tool to determine crop 'viromes', as described for sweetpotato above. The data also provide a valuable resource to design more specific and dedicated diagnostic assays that are fit for use in other settings. Using the sweetpotato virome data, CIP has been working on two particular assays over the last decade: diagnostic tube arrays and Loop mediated isothermal amplification (LAMP) assays.

Tube Arrays

With more than 30 viruses now known to infect sweetpotatoes, it is necessary to perform and combine multiple different assays to be certain that a plant is free of them all. Although sRSA provides an easier alternative, it is still relatively expensive and takes at least 2 weeks from initiation to final result. Thus, there is a scope for a multiplex assay that can detect all known sweetpotato viruses rapidly and with high sensitivity in one assay. To achieve this, CIP has been developing an approach combining multiplex PCR with microarray technology to create a rapid and sensitive assay for known sweetpotato viruses. To provide a cost-effective solution that meets the need for user-friendly processing via conventional lab equipment and high-volume manufacturing capacities that comply with in vitro diagnostic (IVD) regulations, the ArrayTube (AT) platform (Alere Technologies GmbH, Jena, Germany) was selected. This platform consists of a customizable microarray integrated into a 1.5 ml micro tube, which simplifies handling and is used for routing testing in the medical field (Braun et al. 2012; Schneeberg et al. 2015). The AT is printed with custom-designed probes corresponding to regions of the target virus, which are amplified prior to hybridization in the AT by a multiplex PCR reaction with primers corresponding to the same target viruses and a number of controls. PCR fragments are labeled with biotin during amplification, enabling their detection by ELISA after hybridization to the AT. Positive reactions will show up as dots at the corresponding probe position and can be documented and analysed with a dedicated AT reader, or even using a cellphone and a specific App designed by CIP. Design of an effective AT for multiple viruses is complex, requiring design of many primer-probe combinations and several iterations to optimize specificity. In the case of the sweetpotato virus AT that we developed, the sequence data obtained from the African sweetpotato virome project were used to design primers and probes able to detect all common sweetpotato viruses, and the samples were used for their validation. Unfortunately, Alere Technologies recently discontinued the production of the ArrayTube, highlighting the risk of utilizing technologies from a single provider for method development.

Loop-Mediated Isothermal Amplification (LAMP) for Sensitive Field-Based Diagnostics

PCR-based methods have the great benefits of speed and sensitivity, being able to detect minute amounts of a target nucleic acid. However, these are offset by the need for physically large and power-hungry thermal cycling equipment and the need for laboratory conditions for nucleic acid extraction to achieve a sufficiently pure preparation. This combination of requirements makes the technique unsuitable for routine use under field conditions. Over the past 10 years, a number of isothermal amplification methods have been developed using various approaches and their use

in diagnostics is reviewed in Boonham et al. (2013). The benefit of isothermal amplification is its requirement for much simpler equipment to run the reactions, and several field-portable battery-powered options are available (e.g. realtime Genie, Bio-ranger). Some of them are also considerably more robust to contaminants, enabling the use of rapid and simple nucleic acid extraction protocols directly in the field. CIP, together with partners, has been developing assays based on one of these: LAMP for the major sweetpotato viruses, including SPFMV, SPCSV and begomoviruses. LAMP reagents can be pre-loaded and lyophilized into reaction tubes to make them room stable. Extractions are done by macerating leaf punches in an alkaline PEG solution (Chomczynski and Rymaszewski 2006) and using disposable inoculation loops to transfer extract to reaction tubes (in which reagents have been reconstituted in molecular grade water). Assays take anything from 20 min to 1 h to perform depending on the virus titres in the plants and results are displayed in real time. Field testing of these assays, under various climatic conditions in Kenya, has shown them to be robust and reliable in detecting target viruses as compared to PCR (done from the same samples taken back to the lab). Thus, if the price can be reduced sufficiently through volume production, there is potential for such assays to be used in field inspection of certified planting material. Currently inspections are based purely on symptoms and thus can only eliminate SPVD-affected plants, leaving a significant reservoir of viruses in the seed plots, which can combine to form SPVD in production fields.

Lessons Learned and the Way Forward

Under the Sweetpotato for Profit and Health Initiative launched in 2009, CIP and over 30 partners were able to reach 6.2 million households with improved varieties of sweetpotato by July 2019. Along the way, we have been able to test different seed delivery approaches and continue to work on understanding the bottlenecks in the seed system and conducting research to address them. Clearly, addressing the virus issue has been a major focus.

The research described above has elucidated the extent to which different viruses contribute to yield reduction, has shed light on the mechanisms through which they interact, and has described more sensitive and rapid diagnostic methods. It has also suggested novel approaches to virus control through transgenic means. The continental surveys have confirmed the universal importance of SPCSV and SPFMV, but have also revealed several new viruses and viral strains, although most of them are only minor and local in occurrence. Widespread occurrence of novel badnavirus and mastrevirus seem to have only limited relevance since these viruses are not associated with any type of disease symptoms and occur only in very low titres. In contrast, begomoviruses were the third most prevalent viruses in sweetpotato. Because the sweetpotato begomoviruses cause almost imperceptible symptoms they have largely gone unnoticed until recently. Nevertheless, they can cause significant yield losses on their own, even in cultivars considered resistant to SPVD. Thus, breeders

need to start taking begomovirus resistance into account, as do seed producers who will need inexpensive, sensitive and rapid diagnostic methods to be successful. The LAMP assays developed for sweetpotato viruses described above may provide a solution, particularly since they are also semi-quantitative and could enable breeders to detect partial resistance in their material.

Because production of virus-free seed vines is expensive, deploying molecular diagnostics in a sustainable way in sweetpotato seed systems is likely to occur only when a significant proportion of sweetpotato growers become highly commercial in their orientation, or when governments commit to using them to ensure high quality early-generation seed. This is beginning to emerge in some locations, either to provide fresh roots to urban centres in Africa, or to serve the export market to Europe through public-private partnerships. Deploying LAMP-type molecular tests to support resistance breeding may be more straightforward, as the cost of the test would be offset by the increased speed and accuracy of the result. The current approach is visual assessment using a largely subjective scale from 1 to 5 for virus resistance.

At a time when international funding agencies are focusing on short-term impacts and outcomes it is noteworthy that basic and applied virology research has revealed previously unsuspected virus problems in sweetpotato and has helped to provide solutions to them. We propose that continued surveillance of sweetpotato viruses should be conducted to monitor the emergence of new viruses and variants that can be expected as a result of increasing global trade and climate change. Further investment into the largely unknown mechanisms of virus resistance to the different viruses, a truly underinvested area of research, is also needed to assist breeding efforts to develop resistant varieties more efficiently.

Acknowledgements This Open Access book chapter has been made possible by support from the Bill and Melinda Gates Foundation. Research described in this chapter was undertaken as part of, and funded by, the CGIAR Research Program on Roots, Tubers and Bananas (RTB) and supported by CGIAR Trust Fund contributors (https://www.cgiar.org/funders/).

References

Adikini, S., Mukasa, S. B., Mwanga, R. O. M., & Gibson, R. W. (2016). Effects of sweet potato feathery mottle virus and sweet potato chlorotic stunt virus on the yield of sweet potato in Uganda. *Journal of Phytopathology, 164*(4), 242–254. https://doi.org/10.1111/jph.12451.

Aritua, V., Barg, E., Adipala, E., & Vetten, H. J. (2007). Sequence analysis of the entire RNA genome of a sweet potato chlorotic fleck virus isolate reveals that it belongs to a distinct carlavirus species. *Archives of Virology, 152*(4), 813–818. https://doi.org/10.1007/s00705-006-0891-z.

Ateka, E. M., Njeru, R. W., Kibaru, A. G., Kimenju, J. W., Barg, E., Gibson, R. W., & Vetten, H. J. (2004). Identification and distribution of viruses infecting sweet potato in Kenya. *Annals of Applied Biology, 144*(3), 371–379. https://doi.org/10.1111/j.1744-7348.2004.tb00353.x.

Ateka, E. M., Barg, E., Njeru, R. W., Thompson, G., & Vetten, H. J. (2007). Biological and molecular variability among geographically diverse isolates of sweet potato virus 2. *Archives of Virology, 152*(3), 479–488. https://doi.org/10.1007/s00705-006-0879-8.

Baulcombe, D. (2004). RNA silencing in plants. *Nature, 431*(7006), 356–363.

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., Ezzati, M., Grantham-McGregor, S., Katz, J., Martorell, R., Uauy, R., & Maternal, Child Nutrition Study G. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet, 382*(9890), 427–451. https://doi.org/10.1016/S0140-6736(13)60937-X.

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2013). Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research Available on Line, 0*. https://doi.org/10.1016/j.virusres.2013.12.007.

Braun, S. D., Ziegler, A., Methner, U., Slickers, P., Keiling, S., Monecke, S., & Ehricht, R. (2012). Fast DNA serotyping and antimicrobial resistance gene determination of Salmonella enterica with an oligonucleotide microarray-based assay. *PLoS One, 7*(10), e46489.

Chomczynski, P., & Rymaszewski, M. (2006). Alkaline polyethylene glycol-based method for direct PCR from bacteria, eukaryotic tissue samples, and whole blood. *BioTechniques, 40*(4), 454–458.

Clark, C. A., Davis, J. A., Abad, J. A., Cuellar, W. J., Fuentes, S., Kreuze, J. F., Gibson, R. W., Mukasa, S. B., Tugume, A. K., Tairo, F. D., & Valkonen, J. P. T. (2012). Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease, 96*(2), 168–185.

Cuellar, W. J., Tairo, F., Kreuze, J. F., & Valkonen, J. P. T. (2008). Analysis of gene content in sweet potato chlorotic stunt virus RNA1 reveals the presence of p22 RNA silencing suppressor in only few isolates: Implications to viral evolution and synergism. *Journal of General Virology, 89*(2), 573–582.

Cuellar, W. J., Kreuze, J. F., Rajamäki, M.-L., Cruzado, K. R., Untiveros, M., & Valkonen, J. P. T. (2009). Elimination of antiviral defense by viral RNase III. *PNAS, 106*(25), 10354–10358.

Cuellar, W. J., Cruzado, R. K., Fuentes, S., Untiveros, M., Soto, M., & Kreuze, J. F. (2011a). Sequence characterization of a Peruvian isolate of sweet potato chlorotic stunt virus: Further variability and a model for p22 acquisition. *Virus Research, 157*(1), 111–115. https://doi.org/10.1016/j.virusres.2011.01.010.

Cuellar, W. J., De Souza, J., Barrantes, I., Fuentes, S., & Kreuze, J. F. (2011b). Distinct cavemoviruses interact synergistically with sweet potato chlorotic stunt virus (genus Crinivirus) in cultivated sweet potato. *Journal of General Virology, 92*(5), 1233–1243. https://doi.org/10.1099/vir.0.029975-0.

Cuellar, W. J., Galvez, M., Fuentes, S., Tugume, J., & Kreuze, J. (2015). Synergistic interactions of begomoviruses with sweet potato chlorotic stunt virus (genus C rinivirus) in sweet potato (I pomoea batatas L.). *Molecular Plant Pathology, 16*(5), 459–471.

De Souza, J., Fuentes, S., Savenkov, S., Cuellar, W., & Kreuze, J. (2013). The complete nucleotide sequence of sweet potato C6 virus: A carlavirus lacking a cysteine-rich protein. *Archives of Virology, 158*(6), 1393–1396. https://doi.org/10.1007/s00705-013-1614-x.

Di Feo, L., Nome, S., Biderbost, E., Fuentes, S., & Salazar, L. (2000). Etiology of sweet potato chlorotic dwarf disease in Argentina. *Plant Disease, 84*(1), 35–39.

Díaz-Pendón, J. A., & Ding, S.-W. (2008). Direct and indirect roles of viral suppressors of RNA silencing in pathogenesis. *Annual Review of Phytopathology, 46*, 303–326. https://doi.org/10.1146/annurev.phyto.46.081407.104746.

Ding, S.-W., & Voinnet, O. (2007). Antiviral immunity directed by small RNAs. *Cell, 130*(3), 413–426. https://doi.org/10.1016/j.cell.2007.07.039.

FAO. (2017). FAOSTAT. In *Food and Agriculture Organization of the United Nations*. Italy: Rome http://www.fao.org/faostat/en/

Fargette, D., Konate, G., Fauquet, C., Muller, E., Peterschmitt, M., & Thresh, J. (2006). Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology, 44*, 235–260.

Fenby, N., Foster, G., Gibson, R., & Seal, S. (2002). Partial sequence of HSP70 homologue gene shows diversity between West African and East African isolates of Sweetpotato chlorotic stunt virus. *Tropical Agriculture, 79*(1), 26–30.

Fuglie, K., Zhong, L., Salazar, L. F., & Walker, T. S. (1999). *Economic impact of virus-free sweetpototo seed in Shandong, China*. Lima: International Potato Center.

Gibson, R., & Kreuze, J. (2015). Degeneration in sweetpotato due to viruses, virus-cleaned planting material and reversion: A review. *Plant Pathology, 64*(1), 1–15.

Gibson, R. W., Mwanga, R. O. M., Kasule, S., Mpembe, I., & Carey, E. E. (1997). Apparent absence of viruses in most symptomless field-grown sweet potato in Uganda. *Annals of Applied Biology, 130*(3), 481–490. https://doi.org/10.1111/j.1744-7348.1997.tb07676.x.

Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E., & Vetten, H. J. (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology, 47*(1), 95–102. https://doi.org/10.1046/j.1365-3059.1998.00196.x.

Grüneberg, W. J., Ma, D., ROM, M., Carey, E. E., Huamani, K., Diaz, F., Eyzaguirre, R., Guaf, E., Jusuf, M., Karuniawan, A., Tjintokohadi, K., Song, Y.-S., Anil, S. R., Hossain, M., Rahaman, E., Attaluri, S., Some, K., Afuape, S., Adofo, K., Lukonge, K., Karanja, L., Ndirigwe, J., Ssemakula, G., Agili, S., Randrianaivoarivony, J.-M., Chiona, M., Chipungu, F., Laurie, R. S. J., Andrade, M., Fernandes, F. R., Mello, A. S., Khan, A., Labonte, D. R., & Yencho, G. C. (2015). Advances in sweetpotato breeding from 1993 to 2012. In *Potato and sweetpotato in Africa: Transforming the value chains for food and nutrition security*. Wallingford: CAB International.

Gruneberg, W. J., Ma, D., Mwanga, R. O. M., Carey, E. E., Huamani, K., Diaz, F., Eyzaguirre, R., Guaf, E., Jusuf, M., Karuniawan, A., Tjintokohadi, K., Song, Y. S., Anil, S. R., Hossain, M., Rahaman, E., Attaluri, S. I., Some, K., Afuape, S. O., Adofo, K., Lukonge, E., Karanja, L., Ndirigwe, J., Ssemakula, G., Agili, S., Randrianavoarivony, J. M., Chiona, M., Chipungu, F., Laurie, S. M., Ricardo, J., Andrade, M., Rausch Fernandes, R., Mello, A. S., Khan, M. A., Labonte, D. R., & Yencho, G. C. (2015). Advances in sweetpotato breeding from 1992 to 2012. In J. Low, M. Nyongesa, S. Quinn, & M. Parker (Eds.), *Potato and sweetpotato in Africa: Transforming the value chains for food and nutrition security* (pp. 3–68). Wallingford: CABI.

Gutiérrez, D. L., Fuentes, S., & Salazar, L. F. (2003). Sweetpotato virus disease (SPVD): Distribution, incidence, and effect on sweetpotato yield in Peru. *Plant Disease, 87*(3), 297–302. https://doi.org/10.1094/PDIS.2003.87.3.297.

Hagenimana, V., Oyunga, M. A., Low, J. W., Njoroge, S. M., Gichuki, S., & Kabira, J. (1999). *The effects of women farmers' adoption of orange-fleshed sweet potatoes: Raising vitamin A intake in Kenya ICRW/OMNI Research Program* (Vol. Research Report Series 3). Washington, DC: International Center for Research on Women.

Karyeija, R. F., Gibson, R. W., & Valkonen, J. P. T. (1998). The significance of sweet potato feathery mottle virus in subsistence sweet potato production in Africa. *Plant Disease, 82*(1), 4–15.

Karyeija, R. F., Kreuze, J. F., Gibson, R. W., & Valkonen, J. P. T. (2000). Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology, 269*(1), 26–36.

Kashif, M., Pietilä, S., Artola, K., Jones, R. A. C., Tugume, A. K., Mäkinen, V., & Valkonen, J. P. T. (2012). Detection of viruses in sweetpotato from honduras and guatemala augmented by deep-sequencing of small-RNAs. *Plant Disease, 96*(10), 1430–1437. https://doi.org/10.1094/pdis-03-12-0268-re.

Kassanis, B. (1964). Interactions of viruses in plants. In *Advances in virus research* (Vol. 10, pp. 219–255). Waltham: Elsevier.

Kokkinos, C., & Clark, C. (2006a). Interactions among sweet potato chlorotic stunt virus and different potyviruses and potyvirus strains infecting sweetpotato in the United States. *Plant Disease, 90*(10), 1347–1352.

Kokkinos, C. D., & Clark, C. (2006b). Real-time PCR assays for detection and quantification of sweetpotato viruses. *Plant Disease, 90*(6), 783–788.

Kreuze, J., & Fei, Z. (2019). *Dataset for: The African sweetpotato virome*. https://doi.org/10.21223/DOSS60.

Kreuze, J. F., Savenkov, E. I., & Valkonen, J. P. T. (2002). Complete genome sequence and analyses of the subgenomic RNAs of *sweet potato chlorotic stunt virus* reveal several new features for the genus *Crinivirus*. *Journal of Virology, 76*(18), 9260–9270. https://doi.org/10.1128/JVI.76.18.9260-9270.2002.

Kreuze, J. F., Savenkov, E. I., Cuellar, W., Li, X., & Valkonen, J. P. T. (2005). Viral class 1 RNase III involved in suppression of RNA silencing. *Journal of Virology, 79*(11), 7227–7238. https://doi.org/10.1128/JVI.79.11.7227-7238.2005.

Kreuze, J. F., Klein, I. S., Untiveros Lázaro, M., Cuellar Chuquiyuri, W. J., Lajo Morgan, G., Cipriani Mejía, P. G., Ghislain, M., & Valkonen, J. P. T. (2008). RNA silencing-mediated resistance to a crinivirus (Closteroviridae) in cultivated sweetpotato (Ipomoea batatas L.) and development of sweetpotato virus disease following co-infection with a potyvirus. *Molecular Plant Pathology, 9*(5), 589–598. https://doi.org/10.1111/j.1364-3703.2008.00480.x.

Kreuze, J. F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. *Virology, 388*(1), 1–7. https://doi.org/10.1016/j.virol.2009.03.024.

Kreuze, J.F., Perez, A., Gargurevich, M.G., & Cuellar, W. J. (2020). Badnaviruses of sweetpotato: Symptomless co-inhabitants on a global scale. Frontiers in Plant Science, 11, p. 313.

Kwak, H.-R., Kim, M.-K., Shin, J.-C., Lee, Y.-J., Seo, J.-K., Lee, H.-U., Jung, M.-N., Kim, S.-H., & Choi, H.-S. (2014). The current incidence of viral disease in Korean sweet potatoes and development of multiplex RT-PCR assays for simultaneous detection of eight sweet potato viruses. *The Plant Pathology Journal, 30*(4), 416–424.

Li, F., Zuo, R., Abad, J., Xu, D., Bao, G., & Li, R. (2012). Simultaneous detection and differentiation of four closely related sweet potato potyviruses by a multiplex one-step RT. *Journal of Virological Methods, 186*(1–2), 161–166.

Ling, K.-S., Jackson, D. M., Harrison, H., Simmons, A. M., & Pesic-VanEsbroeck, Z. (2010). Field evaluation of yield effects on the U.S.A. heirloom sweetpotato cultivars infected by Sweet potato leaf curl virus. *Crop Protection, 29*(7), 757–765. https://doi.org/10.1016/j.cropro.2010.02.017.

Loebenstein, G., Fuentes, S., Cohen, J., & Salazar, L. (2003). Sweet potato. In *Virus and virus-like diseases of major crops in developing countries* (pp. 223–248). Dordrecht: Springer.

Lovisolo, O., Hull, R., & Rösler, O. (2003). Coevolution of viruses with hosts and vectors and possible paleontology. *Advances in Virus Research, 62*, 325–379.

Low, J., Kinyae, P., Gichuki, S., Oyunga, M. A., Hagenimana, V., & Kabira, J. (1997). *Combating vitamin A deficiency through the use of sweetpotato: Results from phase I of an action research project in South Nyanza, Kenya*. Lima: International Potato Center in collaboration with the Kenya Agricultural Research Institute.

Low, J. W., Lynam, J., Lemaga, B., Crissman, C., Barker, I., Thiele, G., Namanda, S., Wheatley, C., & Andrade, M. (2009). Sweetpotato in Sub-Saharan Africa. In G. Loebenstein & G. Thottappilly (Eds.), *The sweetpotato* (pp. 359–390). Dordrecht: Springer. https://doi.org/10.1007/978-1-4020-9475-0.

Low, J. W., Ball, A.-M., Magezi, S., Njoku, J., Mwanga, R. O. M., Andrade, M. I., Tomlins, K., Dove, R., & van Mourik, T. (2017a). Chapter 7: Sweet potato development and delivery in sub-Saharan Africa. *AJFAND, 17*(2), 11955–11972.

Low, J. W., Mwanga, R. O. M., Andrade, M. I., Carey, E., & Ball, A.-M. (2017b). Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. *Global Food Security, 14*, 23–30.

MacRae, I. J., & Doudna, J. A. (2007). Ribonuclease revisited: Structural insights into ribonuclease III family enzymes. *Current Opinion in Structural Biology, 17*(1), 138–145.

Matthews, R. E. F., & Hull, R. (2002). *Matthews' plant virology*. Houston: Gulf Professional Publishing.

McEwan, M., Namanda, S., & Lusheshanija, D. (2012). *Whose standards matter? Piloting quality declared planting material inspection guidelines for sweetpotato in Lake Zone, Tanzania*. Paper presented at the 16th Triennial Symposium of the International Society for Tropical Root Crops, Abeokuta, Nigeria, 23–28 September 2012.

McEwan, M., Almekinders, C., Abidin, P. E., Andrade, M., Carey, E. E., Gibson, R. W., Naico, A., Namanda, S., & Schulz, S. (2015). Can small still be beautiful? Moving local sweetpotato seed systems to scale in Sub-Saharan Africa. In J. Low, M. Nyongesa, S. Quinn, & M. Parker (Eds.), *Potato and sweetpotato in Africa: Transforming the value chains for food and nutrition security* (pp. 289–310). Oxfordshire: CABI.

Miano, D. W., LaBonte, D. R., Clark, C. A., Valverde, R. A., Hoy, M. W., Hurtt, S., & Li, R. (2006). First report of a begomovirus infecting sweetpotato in Kenya. *Plant Disease, 90*(6), 832–832. https://doi.org/10.1094/PD-90-0832B.

Milgram, M., Cohen, J., & Loebenstein, G. (1996). Effects of sweet potato feathery mottle virus and sweet potato sunken vein virus on sweet potato yields and rates of reinfection of virus-free planting material in Israel. *Phytoparasitica, 24*(3), 189–193. https://doi.org/10.1007/BF02981417.

Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D. C., García, J. A., & López-Moya, J. J. (2016). The P1N-PISPO trans-frame gene of sweet potato feathery mottle potyvirus is produced during virus infection and functions as an RNA silencing suppressor. *Journal of Virology, 90*(7), 3543–3557.

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2003). Incidence of viruses and viruslike diseases of sweetpotato in Uganda. *Plant Disease, 87*(4), 329–335.

Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2006). Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. *Plant Pathology, 55*(3), 458–467. https://doi.org/10.1111/j.1365-3059.2006.01350.x.

Mulabisana, M., Cloete, M., Laurie, S., Mphela, W., Maserumule, M., Nhlapo, T., Cochrane, N., Oelofse, D., & Rey, M. (2019). Yield evaluation of multiple and co-infections of begomoviruses and potyviruses on sweet potato varieties under field conditions and confirmation of multiple infection by NGS. *Crop Protection, 119*, 102–112.

Mwanga, R. O. M., Odongo, B., Niringiye, C., Alajo, A., Kigozi, B., Makumbi, R., Lugwana, E., Namukula, J., Mpembe, I., Kapinga, R., Lemaga, B., Nsumba, J., Tumwegamire, S., & Yencho, C. G. (2009). 'NASPOT 7', 'NASPOT 8', 'NASPOT 9 O', 'NASPOT 10 O', and 'Dimbuka-Bukulula' sweetpotato. *HortScience, 44*(3), 828–832.

Mwanga, R. O. M., Kyalo, G., Ssemakula, G. N., Niringiye, C., Yada, B., Otema, M. A., Namakula, J., Alajo, A., Kigozi, B., Makumbi, R. N. M., Ball, A.-M., Grüneberg, W. J., Low, J. W., & Yencho, G. C. (2016). 'NASPOT 12 O' and 'NASPOT 13 O' sweetpotato. *HortScience, 51*(3), 291–295.

Mwanga, R. O. M., Grüneberg, W. J., Andrade, M. I., Carey, E. E., Low, J. W., & Yencho, G. C. (2017). *Genetic improvement of Sweetpotato, Ipomoea batatas L. (Lam.)*. New York: Springer.

Njeru, R. W., Mburu, M. W. K., Cheramgoi, E., Gibson, R. W., Kiburi, Z. M., Obudho, E., & Yobera, D. (2004). Studies on the physiological effects of viruses on sweet potato yield in Kenya. *Annals of Applied Biology, 145*(1), 71–76. https://doi.org/10.1111/j.1744-7348.2004.tb00360.x.

Njeru, R. W., Bagabe, M. C., Nkezabahizi, D., Kayiranga, D., Kajuga, J., Butare, L., & Ndirigue, J. (2008). Viruses infecting sweet potato in Rwanda: Occurrence and distribution. *Annals of Applied Biology, 153*(2), 215–221. https://doi.org/10.1111/j.1744-7348.2008.00252.x.

O'Brien, P. J. (1972). The sweet potato: Its origin and Dispersal1. *American Anthropologist, 74*(3), 342–365. doi: http://onlinelibrary.wiley.com/doi/10.1525/aa.1972.74.3.02a00070/pdf.

Opiyo, S., Ateka, E., Owuor, P., Manguro, L., & Miano, D. (2010). Development of a multiplex PCR technique for simultaneous detection of sweet potato feathery mottle virus and sweet potato chlorotic stunt virus. *Journal of Plant Pathology, 92*, 363–366.

Pooggin, M. M. (2018). Small RNA-omics for plant virus identification, virome reconstruction and antiviral defense characterization. *Frontiers in Microbiology, 9*, 2779.

Pruss, G. J., Lawrence, C. B., Bass, T., Li, Q. Q., Bowman, L. H., & Vance, V. (2004). The potyviral suppressor of RNA silencing confers enhanced resistance to multiple pathogens. *Virology, 320*(1), 107–120.

Qiao, Q., Zhang, Z.-C., Qin, Y.-H., Zhang, D.-S., Tian, Y.-T., & Wang, Y.-J. (2011). First report of sweet potato chlorotic stunt virus infecting sweet potato in China. *Plant Disease, 95*(3), 356–356.

Rajendran, S., Kimenye, L. N., & McEwan, M. (2017). Strategies for the development of the sweetpotato early generation seed sector in eastern and southern Africa. *Open Agriculture, 2*, 236–243. https://doi.org/10.1515/opag-2017-0025.

Rännäli, M., Czekaj, V., Jones, R. A. C., Fletcher, J. D., Davis, R. I., Mu, L., & Valkonen, J. P. T. (2009). Molecular characterization of sweet potato feathery mottle virus (SPFMV) isolates from Easter Island, French Polynesia, New Zealand, and Southern Africa. *Plant Disease, 93*(9), 933–939. https://doi.org/10.1094/PDIS-93-9-0933.

Rodamilans, B., Valli, A., Mingot, A., San León, D., López-Moya, J. J., & García, J. A. (2018). An atypical RNA silencing suppression strategy provides a snapshot of the evolution of sweet potato-infecting potyviruses. *Scientific Reports, 8*(1), 15937.

Schneeberg, A., Ehricht, R., Slickers, P., Baier, V., Neubauer, H., Zimmermann, S., Rabold, D., Lübke-Becker, A., & Seyboldt, C. (2015). DNA microarray-based PCR ribotyping of Clostridium difficile. *Journal of Clinical Microbiology, 53*(2), 433–442.

Sindi, K., Bocher, T., Low, J. W., & Ndirigue, J. (2015). *Rwanda sweetpotato super foods: Market chains that work for women and the poor. Brief*. Nairobi: Sweetpotato Action for Security and Health in Africa (SASHA) Project.

Smith, K. M. (1931). On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators and selective methods of transmission. *Proceedings of the Royal Society of London Series B, Containing Papers of a Biological Character, 109*(762), 251–267.

Smith, K. (1960). *Plant viruses* (p. 57). London: Methuen & Co. Ltd.

Sommer, A., & West, K. P. (1996). *Vitamin A deficiency: Health, survival, and vision*. Oxford: Oxford University Press.

Stevens, G. A., Bennett, J. E., Hennocq, Q., Lu, Y., De-Regil, L. M., Rogers, L., Danaei, G., Li, G., White, R. A., Flaxman, S. R., Oehrle, S.-P., Finucane, M. M., Guerrero, R., Bhutta, Z. A., Then-Paulino, A., Fawzi, W., Black, R. E., & Ezzati, M. (2015). Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: A pooled analysis of population-based surveys. *The Lancet Global Health, 3*(9), e528–e536. https://doi.org/10.1016/S2214-109X(15)00039-X.

Stukenbrock, E. H., & McDonald, B. A. (2008). The origins of plant pathogens in agro-ecosystems. *Annual Review of Phytopathology, 46*, 75–100.

Szittya, G., Silhavy, D., Molnár, A., Havelda, Z., Lovas, A., Lakatos, L., Bánfalvi, Z., & Burgyán, J. (2003). Low temperature inhibits RNA silencing-mediated defence by the control of siRNA generation. *The EMBO Journal, 22*(3), 633–640. Doi: http://www.nature.com/emboj/journal/v22/n3/suppinfo/7594983a_S1.html.

Tairo, F., Mukasa, S. B., Jones, R. A. C., Kullaya, A., Rubaihayo, P. R., & Valkonen, J. P. T. (2005). Unravelling the genetic diversity of the three main viruses involved in Sweet Potato Virus Disease (SPVD), and its practical implications. *Molecular Plant Pathology, 6*(2), 199–211. https://doi.org/10.1111/j.1364-3703.2005.00267.x.

Tugume, A. K. (2010). *The significance of wild plants in the evolutionary ecology of three major viruses infecting cultivated sweetpotato in Uganda*. Helsinki: University of Helsinki.

Tugume, A., Mukasa, S., & Valkonen, J. (2008). Natural wild hosts of sweet potato feathery mottle virus show spatial differences in virus incidence and virus-like diseases in Uganda. *Phytopathology, 98*(6), 640–652.

Tugume, A. K., Cuéllar, W. J., Mukasa, S. B., & Valkonen, J. P. T. (2010a). Molecular genetic analysis of virus isolates from wild and cultivated plants demonstrates that East Africa is a hotspot for the evolution and diversification of sweet potato feathery mottle virus. *Molecular Ecology, 19*(15), 3139–3156. https://doi.org/10.1111/j.1365-294X.2010.04682.x.

Tugume, A. K., Mukasa, S. B., Kalkkinen, N., & Valkonen, J. P. T. (2010b). Recombination and selection pressure in the ipomovirus sweet potato mild mottle virus (Potyviridae) in wild species and cultivated sweetpotato in the centre of evolution in East Africa. *Journal of General Virology, 91*(4), 1092–1108.

Tugume, A. K., Amayo, R., Weinheimer, I., Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2013). Genetic variability and evolutionary implications of RNA silencing suppressor genes in RNA1 of *Sweet Potato Chlorotic Stunt Virus* isolates infecting Sweetpotato and related wild species. *PLoS One, 8*(11), e81479. https://doi.org/10.1371/journal.pone.0081479.

Tugume, A. K., Mukasa, S. B., & Valkonen, J. P. (2016). Mixed infections of four viruses, the incidence and phylogenetic relationships of sweet potato chlorotic fleck virus (Betaflexiviridae) isolates in wild species and sweetpotatoes in Uganda and evidence of distinct isolates in East Africa. *PLoS One, 11*(12), e0167769.

Untiveros, M., Fuentes, S., & Salazar, L. F. (2007). Synergistic interaction of sweet potato chlorotic stunt virus (Crinivirus) with Carla-, Cucumo-, Ipomo-, and Potyviruses infecting sweet potato. *Plant Disease, 91*(6), 669–676.

Untiveros, M., Olspert, A., Artola, K., Firth, A. E., Kreuze, J. F., & Valkonen, J. P. (2016). A novel sweet potato potyvirus open reading frame (ORF) is expressed via polymerase slippage and suppresses RNA silencing. *Molecular Plant Pathology, 17*(7), 1111–1123.

Vance, V. B., Berger, P. H., Carrington, J. C., Hunt, A. G., & Shi, X. M. (1995). 5′ proximal potyviral sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco. *Virology, 206*(1), 583–590.

Wanjala, B. W., Ateka, E. M., Miano, D. W., Low, J. W., & Kreuze, J. F. (2019). Storage root yield of sweetpotato as influenced by Sweetpotato leaf curl virus and its interaction with Sweetpotato feathery mottle virus, and Sweetpotato chlorotic stunt virus in Kenya. *Plant Disease*. https://doi.org/10.1094/PDIS-06-19-1196-RE.

Wasswa, P., Otto, B., Maruthi, M. N., Mukasa, S. B., Monger, W., & Gibson, R. W. (2011). First identification of a sweet potato begomovirus (sweepovirus) in Uganda: Characterization, detection and distribution. *Plant Pathology, 60*(6), 1030–1039. https://doi.org/10.1111/j.1365-3059.2011.02464.x.

Weinheimer, I., Jiu, Y., Rajamäki, M.-L., Matilainen, O., Kallijärvi, J., Cuellar, W. J., Lu, R., Saarma, M., Holmberg, C. I., & Jäntti, J. (2015). Suppression of RNAi by dsRNA-degrading RNaseIII enzymes of viruses in animals and plants. *PLoS Pathogens, 11*(3), e1004711.

Weinheimer, I., Haikonen, T., Ala-Poikela, M., Moser, M., Streng, J., Rajamäki, M.-L., & Valkonen, J. P. (2016). Viral RNase3 co-localizes and interacts with the antiviral defense protein SGS3 in plant cells. *PLoS One, 11*(7), e0159080.

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D. C., Olukolu, B. A., & Wang, H. (2018). Genome sequences of two diploid wild relatives of cultivated sweetpotato reveal targets for genetic improvement. *Nature Communications, 9*(1), 4580.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

Part III
Global Impacts of Plant Disease Epidemics

Chapter 6
The Impact of Rice Diseases in Tropical Asia

N. P. Castilla, J. B. Macasero, J. E. Villa, A. H. Sparks, L. Willocquet, and S. Savary

Abstract This chapter reviews approaches taken to assessing the impact of diseases on rice crops, primarily in tropical Asia, compared with insect pests and weeds. Published estimates of yield loss show great variation between and within reports. This reflects the diversity of metrics used and differences in method, location, scale, crop ecosystem etc., exposing the inconsistency of the estimates as representative of rice production. Results are then summarised of >1000 surveys of farmers' fields conducted over 25 years to a standardised protocol. Estimates were made of the levels of injury to the growing crop attributed to each disease or pest species present, and of the crop yield. Records of physical, biological, and socio-economic characteristics showed the importance of the influence of the crop's "production situation" on the impact of pathogen and pest species. These surveys were complemented by the results of yield-loss experiments, and by epidemic modelling (based on EPIRICE) and yield-loss modelling (based on RICEPEST). An overview of the results of this research programme is presented, together with a summary of recent trends in rice crop health in Asia. In contrast with views widely held in the 1980s, plant diseases now appear to have a much greater impact on rice production than insect pests.

N. P. Castilla
Adaptive Agronomy and Pest Ecology Cluster, Sustainable Impact Platform, International Rice Research Institute, Los Baños, Philippines
e-mail: n.castilla@irri.org

J. B. Macasero · J. E. Villa
Institute of Weed Science, Entomology and Plant Pathology, College of Agriculture and Food Science, University of the Philippines Los Baños College, Los Baños, Laguna, Philippines
e-mail: J.Macasero@irri.org; jevilla@up.edu.ph

A. H. Sparks
Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD, Australia
e-mail: Adam.Sparks@usq.edu.au

L. Willocquet · S. Savary (✉)
INRAE, UMR AGIR, Castanet Tolosan cedex, France
e-mail: laetitia.willocquet@inrae.fr; serge.savary@inrae.fr

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_6

Introduction

Rice (*Oryza sativa*), the first staple of Asia, is produced in a wide range of agroecological conditions (Greenland 1997), from large river deltas to high elevation paddy systems, and on a broad range of latitudes. Rice is produced in systems also involving wheat, maize, legumes, rapeseed, and potatoes; but rice is also produced in nearly rice-exclusive systems, sometimes with seven harvests in two calendar years. The rice trade crisis of 2008 led to soaring retail prices on markets; the resulting price spike affected foremost the poor, because rice is the staple of roughly half the poor in the world (Zeigler and Savary 2009). Such an event concerns plant pathologists: plant diseases are major yield-reducers. Therefore, improving crop health can significantly improve yield, secure trade, stocks, and markets vital to food security, as well as enhancing the efficiency of use of increasingly scarce and often non-renewable agricultural resources: water, labour, energy, land, and genetic resources.

Changes in cropping practices and systems will inevitably lead to new crop health problems, not only in terms of emerging or re-emerging diseases but also in terms of combinations of yield-reducing factors, including diseases (Zeigler and Savary 2009). This chapter reviews advances that have been made to better characterise the state of health of rice crops and the impact of rice diseases on rice production, especially in Asia. However, the ongoing changes driven by climate and globalisation concern all crops, and we believe many of the concepts and approaches used in rice can have applications for other crops and in other parts of the world.

Information on crop health is the basis for any decision, short- or long-term, in crop health management: this applies to research policies – which priority to give to which research direction; plant breeding programmes – which trait to breed first; plant protection programmes – which disease(s) or pest(s) to control, to what extent, in which environmental, social, technical, and economic context. This chapter describes how a large-scale international crop health assessment was developed for rice, and summarises some of its results.

The Knowledge Background to Crop Health Assessment in Rice

In 1987, Paul Teng convened a very large meeting at the International Rice Research Institute involving over 120 participants to assess the new directions the institute was taking with respect to rice health, and especially with respect to crop losses (Teng 1990a). The meeting was a unique opportunity for scientists all over the world to share ideas, methods, and concepts on the management of plant health and the assessment of crop losses.

Proceedings of this conference indicate that, by 1990, limited data were available to quantify the importance of rice pathogens and pests. The analysis by Cramer

(1967) was then one of the most cited sources, reporting rice yield losses as percentages with the following figures:

- Losses to all insects: 34.4%
- Losses to all diseases: 9.9%
- Losses to weeds: 10.8%
- Potential (in this text: Attainable) production harvested: 44.9%
- Total potential (in this text: Attainable) production lost before harvest: 55.1%

According to Cramer (1967), therefore, more rice was lost to pathogens, pests, and weeds than was harvested. Although these were seen as high figures at the time (Teng et al. 1990), there were few hard facts to contradict them. Cramer's (1967) figures actually conform to the data available then, and some even seemed to be underestimates. With respect to losses to insects, Ahrens et al. (1982) for instance report 23.7% yield losses in East and Southeast Asia, on the basis of over 12 years of experimental data with pesticides. Pathak and Dhaliwal (1981) report 35–44% losses for tropical rice; Way (1976) reports 35% losses in India and 16–30% losses in the Philippines; Alam (1961) reports 6% losses for Bangladesh; Fernando (1966) reports 20% losses in Sri Lanka. Litsinger et al. (1987) had reported 18.3% losses in "non-outbreak" years, i.e., losses caused by chronic insect injuries. With respect to weed infestation, very few data were available and that remains the case. A key reference is Moody (1982), with weed yield losses in the Philippines ranging from 11 to 65%.

The list of figures cited in the synthesis by Teng et al. (1990) is summarised in Table 6.1, which prompts several remarks. First is the long, itemised list of rice diseases and pests (much summarised here); second is the metric used to express losses, here percentages; third is the large variation in loss levels for the same disease or pest among references (i.e., among studies in different locations or periods); and fourth is the variation in losses within a specific reference (i.e., in the same study). It is useful to examine these elements, because they lead to directions for investigation.

The itemised information on crop loss to harmful organisms is explicit in Table 6.1; nowhere was there in the rice scientific literature before 1990 an attempt to address crop loss to harmful organisms as a whole, in part because of disciplinary boundaries. As a result, the itemised list of Table 6.1 conveys a message of disorder and complexity, amplified by the implicit issue of losses to combined disease or pest injuries.

Losses in percent (as in Table 6.1) refer to the FAO definition (Chiarappa 1971; Teng 1987): the difference between the attainable (Ya; dimension: [biomass]), uninjured, yield and the actual (injured) yield (Y; dimension: [biomass]), standardised for the attainable yield: (Ya − Y)/Ya, or relative yield loss, with dimension [biomass.biomass^{-1}] = [1]. However, losses are often also reported in total country harvest loss (dimension: [biomass]), in total economic loss (dimension: [money]), or in agricultural area affected (dimension: [squared length]). Loss reporting at the country scale (or at the scale of any administrative unit) is of interest for economic analysis at the meso-scale, but is of nearly no value for analyses at the field or farm scales;

Table 6.1 List of rice yield-loss estimates from pathogens and insect pests reported by Teng et al. (1990)

Disease or pest	Scientific name	Country	Yield losses reported	Reference
Bacterial blight	*Xanthomonas oryzae*	India	6–60%	Srivastava (1967)
		China	4.9–6%	Teng (1986)
Brown spot	*Cochliobolus miyabeanus*	Bangladesh – East India (1942–1943)	80%	Padmanabhan (1973)
		India	14–41%	Vidhyasekaran and Ramados (1973)
Rice blast	*Pyricularia grisea*	India	5–10%	Padmanabhan (1965)
		China	8.4–14.0%	Teng (1986)
		Philippines	50–60%	Nuque (1963)
		Philippines	70–85%	Nuque (1970)
Sheath blight	*Rhizoctonia solani*	Philippines	7.5–22.7%	Ou and Bandong (1976)
		China	9.1–12.6%	Teng (1986)
Stem rot	*Magnaporthe salvinii*	India	5–10%	Chauhan et al. (1968)
		Philippines	30–80%	Hernandez (1923)
Tungro	Rice tungro bacilliform virus and Rice tungro spherical virus	Malaysia	1%	Heong and Ho (1987)
		Bangladesh	40–60%	Reddy (1973)
		Thailand	50%	Wathanakul and Weerapat (1969)
Gall midge	*Pachydiplosis oryzae*	India	12–35%	Reddy (1967)
		Vietnam	50–100%	Reddy (1967)
Leaf and plant hoppers	*Recilia dorsalis; Nilaparvata lugens; Sogatella furcifera*	Bangladesh	50–80%	Alam (1967)
		India	1.1–32.5%	Jayaraj et al. (1974)
Leaffolder	*Cnaphalococris medinalis*	India	50%	Balasubramaniam et al. (1973)
Rice bugs	*Leptocorisa spp.*	India	10%	Pruthi (1953)
Rice hispa	*Dicladispa armigera*	Bangladesh	10–65%	Barr et al. (1975)
		India (Bihar)	50%	Barr et al. (1975)
Stem borers	*Scirpophaga incertulas; S. innotata; Chilo suppressalis; Sesamia inferens*	Bangladesh	30–70% (outbreak)	Alam et al. (1972)
			3–20% (non-outbreak)	
		India	3–95%	Ghose et al. (1960)
		Indonesia	Up to 95%	Soenardi (1967)
		Malaysia	35%	Wyatt (1957)

loss reporting in area affected is of value for administrative purposes, but has very little economic value, and nearly none biologically. Yield loss reporting at the field scale as relative yield losses (nearly always as %) is now the international standard (Chiarappa 1971), enabling comparisons between seasons, harmful organisms, or geographical areas. While useful from an economic or agronomic standpoint at the field scale, this metric the relative, field scale, yield-loss information raises two groups of questions. One is the upscaling of loss figures from the field scale to broader scales, to enable economic analysis; the other is the reference yield (the "unharmed", attainable yield Ya) used. This is because the attainable yield is a highly sensitive and variable, key marker of a given production situation (Zadoks and Schein 1979; Teng 1987; Savary et al. 2017).

The existence of large variation of yield loss estimates, between and within reports, exposes the fundamental issue of representativeness of estimates (Savary et al. 1998). The representativeness of yield loss reports in rice to diseases was analysed, based on published studies involving different groups of diseases (viral, bacterial, and fungal), and considering different rice production systems. Four criteria of representativeness were used: (1) over time (one or more crop cycles considered), (2) over space (one or more locations considered), (3) of scale (size of plant populations considered – pots, to plots to fields), and (4) of injury (the standard deviation of the proportion of studies using inoculation, spontaneous infection, or chemical control). A strong imbalance among disease types was found. Most of the few studies on yield loss due to viral diseases (mainly rice tungro disease) were conducted on the individual (potted) plant scale, and on one-year data sets, often reflecting very severe epidemics only. All studies on bacterial diseases were conducted in single locations only. The vast majority of reports pertained to the irrigated rice ecosystem, and very few addressed the upland, rain-fed lowland, or deep-water rice ecosystems; most reports on irrigated rice refer to one year and one location. In summary, a very large fraction of the available yield loss data available before 1990 was subject to some representativeness bias, explaining the wide differences in reported loss estimates.

Four main research directions were followed to address the needs for better crop health assessment and better yield loss measurement in rice:

1. The development of a large-scale survey in farmers' fields, using an international protocol;
2. The establishment of an experimental crop loss database;
3. The development of a process-based simulation platform for rice disease epidemics;
4. The development of a yield-loss simulation modelling structure.

These four directions are addressed below, with two parts each. One, more detailed, concerns the conceptual and methodological basis which has been implemented: this research on rice has application for several other crop systems. The other summarises some of the key results achieved. In the last section, we address:

5. The recent changes of crop health in rice across Asia.

Concepts, Methods, and Approaches to Assessing Plant Health – Rice in Asia

Concepts and Methods to Implement Surveys in Farmers' Fields

Survey Protocol: Concepts and Principles

Among the many bottlenecks of integrated pest management (IPM) (Jeger 2000), one is the quantity and diversity of information to be collected and shared. A strategy is required, with clear objectives, and description of the various instruments designed to meet these objectives. Using an earlier base developed at the International Rice Research Institute (Elazegui et al. 1990), a "Survey Portfolio" for the assessment of crop health in farmers' fields (Savary et al. 1996) was designed with the following specifications in its practical use:

1. To provide accurate information on the state of plant health in a population of farmers' fields, on the basis of information collected at the individual farmer's field scale;
2. To focus on those components of the rice crop ecosystems that are critical to assess the state of plant health (diseases, animal pests, weeds), and the conditions of crop growth and development;
3. To be practically feasible, including only those attributes that must be qualified or quantified, using effective, rapid, and shareable procedures;
4. To constitute an actual portfolio for action, with three elements: the field procedures themselves; the principles underpinning these procedures; and an explicit outline of how the collected data are to be analysed; and
5. To be congruent with, and approved by, the multiple institutions and colleagues in several countries who are involved in the collection of data, their analysis, and their reporting.

Use of the Survey Portfolio was meant to meet four objectives: (1) to achieve a reasonable description of production situations, including a characterisation of the patterns of cropping practices, and of the set of environmental factors determining the actual yield (Y) of a rice crop; (2) to characterize the combinations of pests and the associated injuries that may occur in any particular field; (3) to enable the establishment of links between production situations and injury combinations; and (4) to enable generation of a measure of the links between production situations, injury combinations, and variation in actual yield.

The design of the Survey Portfolio thus included two major components in data acquisition. A first component deals with the characterisation of the production situation; and a second component deals with the quantification of (i) injuries by harmful organisms (pathogens, animal pests, weeds) and (ii) actual harvested crop yield (Y).

The Survey Portfolio for the Characterization of Rice Pest Constraints (Savary et al. 1996) -- the principles, the collection, and analysis of crop health data -- is based on a few concepts:

1. *Production situation.* The concept of production situation was introduced by De Wit and Penning de Vries (1982) to describe the set of factors -- physical, biological, and socio-economic -- that determine agricultural production. The concept is operationalized (Zadoks 1972) through the inclusion of key components of rice crop management (reflecting the physical and socio-economic environments and their interactions) and a series of rice pests (insects, pathogens, and weeds).
2. *Injury, damage, and loss.* An injury is the visible, measurable result of the biological activity of a pest. An injury may, or may not, translate into damage i.e., yield reduction; this yield reduction may, in turn, translate into loss, often measured in economic terms (Zadoks and Schein 1979; Zadoks 1985). The crop-physiological consequences of a given level of injury depend on the development stage of a crop stand when this injury occurs (Teng and Gaunt 1980): this is because injuries affect the many physiological processes taking place in a growing crop stand. Because crop development governs these processes, measurement of injury must therefore be made at specified crop development stages.
3. *Potential, attainable, and actual yields.* The definitions developed by FAO (Chiarappa 1971) and in Theoretical Production Ecology (Van Ittersum and Rabbinge 1997) are used. The potential yield (Yp) that can be produced by a given genotype of a crop under optimal environmental conditions is in practice limited by a number of factors in a farmer's field: the supply of water or nutrients may not coincide with the needs of the plants at each particular development stage. This resulting attainable yield (Ya) may further be reduced by environmental factors such as storms or diseases and pests. The result is the actual yield (Y) that can be measured in a farmer's field.
4. *Excess of details.* Observations must be justified by the objectives. A survey is not an epidemiological experiment, nor an agronomy study. As in any well designed experimental protocol, each element included in the protocol must have its use. Additional, superfluous elements are costly or time-consuming, and may jeopardise the overall effort.
5. *Precision vs. accuracy.* The two notions, accuracy and precision, are in balance. Accuracy, not precision, is the objective of observations at the field scale. Idealised measurements should be both precise (i.e., as consistent as possible over successive samples) and accurate (i.e., as close as possible to the "true" value) (Forbes and Jeger 1987). However, the limits to the precision of some measurements are very real: for instance, standard deviations of approximately 20% are the norm in surveys on cereals (Church and Austin 1983). In the context of a survey, the unnecessary accumulation of observations is counterproductive: accuracy, not precision, has been shown to decline sharply with the number of observations. In the overall cost of characterisation research, the time of field observation is by far the most expensive, and requires the scarcest resources

(experienced and trained observers are few). Accuracy must come first, and is best achieved with comparatively few observations, which in turn can be distributed over a large number of fields so as to increase representativeness of the survey.

Survey Protocol: Development

1. A detailed field survey procedure is established.
2. This procedure is used in one reference site for a series of cropping seasons.
3. A set of analytical techniques is tested to (a) exploit the acquired information, and (b) select statistical approaches that best fulfil the objectives of the characterisation work.
4. The field survey procedure is then revised and simplified in view of (a) its use in new sites, (b) reliance on fewer observers, and (c) different field observers.
5. The analytical techniques are reviewed so as to best address objectives both on site-specific data sets as well as on combined data sets reflecting different sites.

Survey Protocol: Pattern Analysis with Non-parametric Statistical Methods

The heart of the analysis of survey data consists of successive steps:

- The time-dependent, quantitative information pertaining to pests (diseases, insects, and weeds) is integrated over crop development (not crop growth) to account for the effects of injuries on the crop's physiology;
- Classes reflecting the various distribution frequencies are developed, and the quantitative information is categorised accordingly;
- A (limited; see, e.g. Savary et al. 1994) set of patterns of cropping practices (PR), along with another, independent, set of injury profiles (IE) are characterised from two independent cluster analyses using a chi-square distance (Benzécri 1973a; Wilkinson et al. 2007);
- Two contingency tables, actual crop yield by cropping practices (Y x PR) and actual yield by injury profile (Y x IE), are built and jointly submitted to correspondence analysis (Benzécri 1973b; Hill 1974; Greenacre 1984).

Survey Protocol: Risk Factor Analysis with Parametric Statistical Methods

Public health epidemiologists have documented the relations between individual ways of life and their associated human health risks in many instances. Predicting risk factors from individual habits (i.e., specific elements of ways of life) is widely used in human disease prevention and research prioritisation (Breslow 1978; Willett

2002). Similarly, crop health risks arise from individual diseases, as well as in their combinations, and the environmental contexts where these risks develop may be addressed as risk factors (Savary et al. 2011).

Logistic regression (Agresti 2002; Harrell 2001) models may be used to assess risk factors at different levels of hierarchy (Allen and Starr 1982). These levels of hierarchy are: (a) the production situations (PS), and (b) the components of the production situation, on the one hand, and (i) the injury profile and (ii) the individual injury (disease, animal pest, weed) components, on the other hand. A series of logistic regression models are developed and tested, as:

$$\ln\left[P\left(IP = IP_i\right)/\left(1 - P\left(IP = IP_i\right)\right)\right] = \alpha_{1i} + \sum \beta_{ij} PS_j$$

to quantify the likelihood of association between the occurrence of injury profile IP_i (i.e., $IP = IP_i$) and the occurrences of the *j* observed production situations ($PS = PS_j$); this represents the first level of hierarchy: [PS × IP];

$$\ln\left[P\left(IP = IP_i\right)/\left(1 - P\left(IP = IP_i\right)\right)\right] = \alpha_{2j} + \sum \gamma_{ik} CP_k$$

to quantify the likelihood of association between injury profile IPi occurrence (IP = IP*i*) and the occurrences of the *k* observed components of production situations (CP = CP*k*); this represents a link between a second level of hierarchy (individual components of production situations), with a first level of hierarchy (injury profiles): [CP × IP];

$$\ln\left[P\left(D = D_l\right)/\left(1 - P\left(D = D_l\right)\right)\right] = \alpha_{3l} + \sum \delta_{il} PS_j$$

to quantify the likelihood of association between the occurrence of disease (pest) injury Dl (i.e., D = Dl) and the occurrences of the *j* observed production situations (PS = PS*j*); this represents the link between a second level of hierarchy (individual components of injury profiles) with a first level of hierarchy (production situations): [PS × D]; and

$$\ln\left[P\left(D = D_l\right)/\left(1 - P\left(D = D_l\right)\right)\right] = \alpha_{4l} + \sum \delta_{lk} CP_k$$

to quantify the likelihood of association between the occurrence of disease (pest) injury Dl (i.e., D = Dl) and the occurrences of the *k* observed components of production situations (CP = CP*k*); this represents the second level of hierarchy, with respect to individual (i.e., specific injuries) components of injury profiles and individual components of production situation: [CP × D].

All analyses were performed with the LOGISTIC procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) and SYSTAT (version 11; San Jose, CA) (Steinberg and Colla 2007).

Concepts and Methods for Yield Loss Experiments

Quantification of yield loss requires measurements of attainable yield (Y). Measurement of attainable yield (the level of yield in absence of any injury caused by any harmful agent), however, is next to impossible under farmers' field conditions. Measurement of both attainable and actual yield, so that the gap — the yield loss due to pathogen or pest injury — is measured, requires experimentation. The level of yield loss depends on (i) whether one or several pathogens or pests are considered, and (ii) the conditions under which crop growth takes place. Specifically, two types of factor interactions are important to consider in multiple-injury yield-loss studies. First, interactions may occur among injuries in their yield-reducing effects. Second, interactions may also occur between injuries and attainable yield: the same level of injury may lead to different levels of losses, depending on production situations, and therefore, on the level of attainable yield. Conversely, the same level of loss may be caused by different levels of a given injury depending on production situations. Crop-loss experiments should thus cover a wide range of attainable yields, as well as a wide range of each of the considered injuries (Shane and Teng 1987; Teng 1987) in order to address these interactions. Experiments become far more extensive when multiple injuries (several pathogens or pests) are considered.

A chain of 11 factorial, unreplicated experiments (Savary et al. 2000b) was conducted in farmers' fields and at the Experimental Farm of the International Rice Research Institute from 1991 to 1995, using a standardized protocol (Savary et al. 1997a). These experiments considered a range of two groups of factors: the injuries caused by rice pests (pathogens, insects, and weeds), and the components of production situations (fertiliser input, water supply, crop establishment method, variety, in different seasons and years leading to different climatic conditions). Each injury treatment included a non-injured reference, thereby enabling the measurement of attainable yield and yield losses. The factors (production situation components, injuries) were chosen to represent lowland rice production situations characterised in surveys conducted in tropical Asia, along with their corresponding range of attainable yield. These experiments complemented one another in exploring the response surface (Teng and Gaunt 1980; Shane and Teng 1987) of rice yields to yield-limiting and yield-reducing factors. The resulting experimental data base consisted of 445 individual plots and involved 11 manipulated injuries in a range of attainable yields of 2–11 t ha^{-1}. Data analysis involved two approaches.

- First, analysis of contingency tables (on ordinal and categorised continuous variables) and correspondence analysis (Benzécri 1973b; Greenacre 1984) were used to map the multivariate relations between injury levels, components of production situation, and yield levels.
- Second, principal component regression (Draper and Smith 1981) of measured actual yield (or yield losses, Ya − Y) on (i) attainable (uninjured) yield, (ii) principal components of injury levels, and (iii) their interaction was conducted. This latter approach generates parametric statistical models allowing the comparison of factors (attainable yield, injury levels, their interactions) on actual yield or

yield losses. It also enables back-computations, revealing the shape of damage functions, i.e., the variation of yield losses as a function of injury levels at varying levels of attainable yield.

Concepts and Methods for Generic Epidemiological Modelling

Plant diseases differ widely in their epidemiological patterns, as a result of the diversity of pathogen life cycles and in response to varying environmental (climate) conditions, crop management, and levels of disease control. One useful term of reference is the notion of potential epidemic (Savary et al. 2012), which is an epidemic where no instrument for disease reduction (e.g., host-plant resistance, pesticides) is being used. Comparing potential epidemics in different diseases, and assessing where potential epidemics can be strongest can provide insight into the reasons why disease distribution may vary across geographical areas and environmental contexts.

The generic epidemiological model EPIRICE (Savary et al. 2012) was developed as a general model framework to address any rice (or plant) disease. The structure of the model was designed to remain as simple as possible, in order to ensure its general value for a number of different pathosystems, and to enable its linkage with other applications, especially geographic information systems. The structure of EPIRICE is centred on a "suscept-exposed-infectious" (Zadoks 1971) shell, which represents the dynamics of the host population under disease. This structure is however flexible enough to capture some of the key epidemiological features of each disease and to allow further improvements.

Five rice diseases were chosen to develop EPIRICE: brown spot, leaf blast, bacterial blight, sheath blight, and rice tungro disease. This choice is based on the reported importance (frequency, losses) of these diseases, and also because these diseases differ with respect to the pathogens involved, the hierarchy (Allen and Starr 1982) of plant structure levels they affect, and their epidemiological characteristics:

- Diseases of foliage causing discrete lesions: brown spot (Dasgupta and Chattopadhyay 1977; Chakrabarti 2001; Pannu et al. 2005) and leaf blast (El-Refaei 1977; Teng 1994a) cause lesions on the leaf blades, which in turn produce propagules that spread the disease.
- Diseases of foliage with extensive, spreading leaf lesions: bacterial blight (Mew 1987; Kauffman and Kannaiyan 1987) also causes lesions on the leaf blades, which expand rapidly in the case of compatible host-pathogen interactions, causing the entire leaf to die, and which are also a source of inoculum for disease spread.
- Diseases of the aerial plant tissues with rapidly expanding lesions on sheaths and leaves: sheath blight is a (primarily) soilborne disease, which affects entire tillers (Castilla et al. 1996). Unlike the previous diseases, the sheath blight pathogen

does not produce propagules per se; instead hyphal strands of the fungus progress over the growing canopy, causing canopy-borne epidemics (Savary et al. 1997b).

- Systemic (whole plant) disease: rice tungro disease is caused by two different viral species, and infection by both the viruses is necessary to cause disease; the viruses are semi-persistently transmitted by the green leafhopper (GLH; Azzam and Chancellor 2002).

Thus, these five diseases affect a hierarchy of plant tissues in a growing crop canopy: fractions of leaf areas (brown spot and leaf blast), entire leaves (bacterial blight), entire tillers (sheath blight), and entire plants (tungro). The dynamics of each of the five diseases therefore inherently depend on the differing nature of sites (i.e., entities which, when occupied by the pathogen may become infectious, Zadoks 1971) to be considered. The sites to be considered are: fractions of leaf area (brown spot and leaf blast), leaves (bacterial blight), tillers (sheath blight), or entire plants (tungro). The way disease is measured in the different pathosystems also differs: severity (% leaf area affected) for brown spot and blast, incidence at the leaf level (% leaves diseased) for bacterial blight, incidence at the tiller level (% tillers diseased) for sheath blight, and incidence at the plant level (% plants infected) for tungro.

The modelling structure incorporates a very simple representation of crop growth (modelled in terms of site numbers that are congruent with the considered disease), of epidemic onset, of residence times (infectious and latent periods), and of intrinsic infection rate. The model also incorporates the effects of daily temperature and canopy wetness, of the age of plant tissues on infection with the plant development stage, and of lesion aggregation (Savary et al. 2012). The model was parameterised using experimental data from the literature on each of the five diseases. Model evaluation was conducted against reference observed epidemics documented in the literature for each of the five diseases, with two approaches, chi-square tests between (categorised) observed (Sokal and Rohlf 1981) and simulated disease levels, and ordinary least-square regression (Teng 1981).

Concepts and Methods for Generic Yield Loss Modelling

Crop yield losses (YL) result from the dynamic interactions between agrophysiological processes and damage mechanisms. The former translate into levels of attainable yield (Ya), which are reflections of shifting production situations (PS). The latter depend on the biological diversity of harmful organisms leading to injury profiles (IP), which also depend on PS.

The process-based yield-loss simulation model for rice, RICEPEST, was designed to simulate rice crop growth and development along with injury mechanisms due to pathogens, insects, and weeds. The model allows simulating crop

growth and yield under the effects of single or multiple injuries, and thus, the yield losses caused by pathogens, pests, and weeds in a range of production situations.

The specifications for model development (Willocquet et al. 1998, 2000) were as follows:

- Provide a simple, process-based simulation of crop growth using the RI-RUE (Monteith 1977) concept: the green tissue of a growing crop stand enables radiation interception (RI); this energy is converted into biomass growth according to the radiation use efficiency (RUE) of the considered crop;
- Account for crop development with a few development stages, especially with two phases: vegetative (pre-anthesis) and reproductive (post-anthesis);
- Account, where needed, for plant structure -- this element was implemented in consideration of the tiller number – panicle number feature in order to account for stem borer injuries;
- Incorporate injury mechanisms that can be applied to any growth-reducing organism (pathogen, animal pest, weed): (1) stand reducers, (2) photosynthetic rate reducers, (3) leaf senescence accelerators, (4) light stealers, (5) assimilate sappers, (6) tissue consumers, and (7) turgor reducers.

The structure of the model is meant to be transparent and generic. It is therefore simple, flexible, and involves as few parameters as possible. The model consists of two linked components: a main component for the dynamics of the rice crop, with accumulation of biomass and its daily partitioning towards leaves, stems, roots, and panicles, and a complementary component for the dynamics of tillering, tiller maturation, panicle formation, and tiller death. RICEPEST incorporates coupling functions representing damage mechanisms due to *Xanthomonas oryzae* pv. *oryzae* (bacterial leaf blight, BLB); *Rhizoctonia solani* (sheath blight, SHB); *Bipolaris oryzae* (brown spot, BS); *Sarocladium oryzae* (sheath rot, SHR); *Pyricularia oryzae* (leaf blast, LB, and neck blast, NB); several species of stem borers (*Scirpophaga incertulas*, *S. innotata*, *Chilo suppressalis*, *Sesamia inferens*, causing white heads, WH, and dead hearts, DH); *Nilaparvata lugens* (brown planthoppers, BPH); various leaf-feeding insects (*Cnaphalocrocis medinalis* and *Hydrellia philippina*, for example); and a range of weeds (WEED), either growing under the rice crop canopy (such as *Monochoria vaginalis*), or above it (such as *Echinochloa* spp., *Cyperus* spp., and *Leptochloa chinensis*). These coupling functions were derived from published and experimental data (Willocquet et al. 1998, 2000, 2002, 2004).

Specific field experiments were designed and implemented at various locations (Vietnam, India, China, and the Philippines) in model development, with two purposes: the parameterisation of (attainable) crop growth, and model evaluation (Willocquet et al. 1998, 2002). Field experiments included statistical main-units, each representing specified production situations (and a corresponding attainable yield). Each main-unit included series of micro-fields (Zadoks and Schein 1979, Savary et al. 2000b), where levels of injuries (diseases, animal pests, weeds) were implemented (Savary et al. 2000b, Willocquet et al. 1998, 2000). Injury-free plots and injured rice plots (with injuries from one or several combined diseases, pests, or weeds or in combination) were established in each main-unit. Crop growth

(including final yield), environmental factors, and injuries were monitored throughout the crop cycle. Data from uninjured plots, i.e., plots that were free of any injury (disease, insect, weeds), were used to calibrate parameters for attainable yield simulation. Data from injured plots were then used to compare simulated and observed yield losses.

Some Results of the Rice Health Assessment

Surveys in Farmers' Fields: Some Results

A total of 1051 individual farmers' fields were surveyed between 1987 and 2011, covering 14 sites across Asia (from India to the Philippines, and from China to Indonesia; Savary et al. 2014). The same survey protocol was employed throughout this entire population of fields, enabling characterisation of each field by 60 different variables describing the crop management, the levels of injury across development stages, and measuring the actual yield from direct 1 m^2 crop cuts. The survey period represents two phases in agricultural development across the region: the full establishment of the Green Revolution (1987–1998), and the Post-Green Revolution (2009–2011). The first phase is characterised by the deployment of high-yielding varieties, of (standardised and limited) subsidies to fertilisers, by the expansion of irrigation systems, by strong centralised agricultural support systems, and by stable, moderate or low commodity prices on the global markets. The second phase is characterised by the deployment of rice hybrids along with high-yielding varieties, by the privatisation of agricultural supply systems (notably, the seed supply, along with the breeding programmes), by the decline of centralised agricultural support systems, by a decline of public investments in agricultural infrastructures (notably, irrigation systems), by an increase of regional and global demand for agricultural commodities, along with high volatility of prices in the national, regional and global markets, and by the first signs of climate change, and perhaps environmental pollution (Savary et al. 2014).

Patterns of Association of Production Situations and Injury Profiles

The main results of the survey in farmers' fields across Asia can be summarised as follows (Savary et al. 2000a, 2006):

1. Patterns of cropping practices that are common across sites can be identified;
2. Similarly, injury profiles that are common across sites can be determined;
3. Patterns of cropping practices and injury profiles are strongly associated at the regional scale;
4. Weather patterns are strongly associated with patterns of cropping practices and injury profiles;

5. Patterns of cropping practices and injury profiles allow for a good description of the variation in actual yield; and
6. Patterns of cropping practices and of injury profiles provide a framework that accurately reflects weather variation and site diversity, and reliably accounts for actual rice yield variation.

The mean estimated yield across sites (4.12 ton.ha^{-1}) corresponds to commonly cited averages in the region, and indicates the potential for progress in productivity. Injury profiles (IN) in farmers' fields were dominated by stem rot and sheath blight (IN1); by bacterial leaf blight, plant hoppers, and leaf folder (IN2); and by sheath rot, brown spot, leaf blast, and neck blast (IN3). IN1 was associated with comparatively high (mineral) fertiliser inputs, long fallow periods, relatively low pesticide use, and good water management in (mostly) transplanted rice crops of a rice-rice rotation. IN2 was associated with direct-seeded rice crops in an intensive rice-rice rotation, where fertiliser and pesticide inputs are low and water management is poor, or where fertiliser and pesticides inputs are high and water management is adequate. IN3 corresponds to low (fertilisers and pesticides) input, labour intensive (hand weeding and transplanting) rice crops in a very diverse rotation system where water supply is uncertain. Weed infestation was an omnipresent constraint across the region.

Production Situations and Their Components as Crop Health Risk Factors

In lowland rice in Asia, highly variable production situations (PS) and their components (technology shifts, labour availability, water supply, mineral fertilisers, pesticide use, and varieties) correspond to large differences in attainable yields (Ya; increases or decreases), and very different injury profiles. However, and critically important, is the fact that these PS x IP (production situation by injury profile) combinations appear to translate into similar yield losses.

Bayesian analysis of survey data, represented by logistic regressions (Savary et al. 2011), shows that (i) production situations, in their entirety, represent very large risk factors (positive or negative) for occurrence of disease syndromes; (ii) production situations are strong risk factors for individual diseases; (iii) drivers of agricultural change represent strong risk factors for disease syndromes; and (iv) drivers of change, taken individually, represent small but significant risk factors for individual diseases. This last analysis indicates that different diseases are positively or negatively associated with shifts in these drivers.

Briefly, this analysis focusing on disease injury profiles (Sheath blight, SHB; Sheath rot, SHR; Brown spot, BS; Leaf blast, LB; Neck blast, NB; and Bacterial leaf blight, BLB) generated five main disease injury profiles (DP):

DP1, with high SHB;
DP2, with high BS, LB, and BLB;
DP3, with high SHB, SHR, BS, LB, and NB;
DP4, with high SHB, SHR, and BS;
DP5, with high BS.

Six production situations were also identified:

PS1, mostly transplanted, with little water shortage, fairly high fertiliser inputs, and long fallow period;
PS2, only transplanted, limited water shortage, moderate fertiliser inputs, and very long fallow period;
PS3, mainly transplanted, serious water shortage, moderate fertiliser inputs, and moderate fallow period;
PS4, only transplanted, limited water shortage, very high fertiliser inputs, and very short fallow period
PS5, mainly direct-seeded, moderate water shortage, fairly high fertiliser inputs, and moderate fallow period, and
PS6, only direct-seeded, moderate water shortage, high fertiliser inputs, and very short fallow period.

A last group of fields, "noclassPS", which do not belong to any of PS1-6, and for which (production situation) variables generally take median values, was created. "noClassPS" is thus neutral in its position relative to other PSs.

At the highest level of organisation hierarchy, risks of given disease injury profiles were very strongly associated with production situations: for instance, there were 85- and 22-fold increases in the odds of syndrome 1 occurring in PSs 1 and 4, respectively, relative to unclassified fields (i.e., the "noclassPS" category, used as the control group). At a lower level of hierarchy (components of production situation x disease injury profile), risk of DP1 occurrence was positively associated with transplanted rice, low water shortage, increased mineral fertilisers, and long fallow periods; DP2 occurred more often under very short fallow period durations (odds ratio = 34.2); and DP3 was associated with intense water shortage, and also with both transplanted rice and low mineral fertiliser input. At the same level of hierarchy, considering the association between individual diseases and production situations, SHB risk was positively and strongly associated with PS4, whereas BS risk was associated with PS3 and PS6. Risks for individual diseases were also often negatively associated with PSs; for example, SHR in PS1, PS4, PS5, and PS6.

The odds ratio found at the lowest level of organisation hierarchy were much lower than at the higher ones, indicating that any individual components of PSs had a comparatively limited predictive ability for individual epidemic risks. Depending on the disease, different PS components predicted the risk of disease. For SHB, crop establishment method and level of mineral fertiliser were significant ($P < 0.05$): direct-seeded rice decreased the SHB epidemic risk by a factor of 5.3, while it was increased by 2.1 with increments of mineral fertiliser of 150 $kg.ha^{-1}$. SHR risk decreased with direct seeded rice and increased with water shortage. BS and LB had similar patterns of risk variation: an increase with water shortage and a decrease with higher fertiliser input and longer fallow periods. Risk of NB was increased by water shortage and decreased in direct-seeded rice and with long fallow periods. BLB risk increased in direct-seeded rice and decreased with water shortage and with long fallow periods.

Overall, results from analysis of farmers' field survey data lead to the main conclusion that domains for pest management strategies (Teng 1990b, 1994b) can be derived from the characterisation of production situations. This work demonstrates that crop health management strategies should be based on production situations that (1) may co-exist at the same geographical location, and (2) may be found at sites thousands of kilometres apart (Savary et al. 2000a, 2006, 2014).

The analysis of crop health risk factors further indicates that the approach has value. The concept of crop health risk factor, and the associated methodologies, could be used for (1) the deployment of technologies, especially host-plant resistances, and (2) production-situation-specific management strategies (Savary et al. 2011).

Yield Loss Experiments: Some Results

A first, nonparametric, multivariate analysis led to a hierarchy of potential injuries, from marginally harmful (e.g., bacterial leaf blight) to extremely harmful (e.g., rice tungro disease). A second, parametric, multivariate approach resulted in a multiple regression model involving factors generated by principal component analysis on injuries that adequately described the variation in actual yield. The principal component regression model has the following shape:

$$Y = aYa + \Sigma b_i F_i + \Sigma c_j Ya \times F_j + \Sigma\Sigma d_{k,l} F_k \times F_l + \varepsilon$$

where

Y and Ya are the actual (injured) and attainable (uninjured) yields,
F_i, F_j, F_k, and F_l represent principal components of disease, pest, and weed injuries,
a, b_i, c_j, and d_k are parameters (parameter vectors), and
the $c_j Ya \times F_j$ and the $d_{k,l} F_k \times F_l$ terms account for interactions between attainable yield and injury factors, and among injury factors, respectively (Savary et al. 2000b).

Principal component analysis on injury variables yielded a series of 11 factors F_i, totalling 100% variance explained. These factors constitute independent linear combinations of the (normalised) injury variables (Draper and Smith 1981). These factors were used in the stepwise multiple regression analysis of the variation in actual yield, Y, using the F_is, the variation in attainable yield (Ya), and their interactions. Overall, a very good description of the variation in actual yield Y was achieved, with an R^2 value of 97.8% and a Fisher ratio of 2269. Examination of residuals (Savary et al. 2000b) did not suggest that hypotheses pertaining to the errors associated with this regression are violated.

A major finding is that some of the [attainable yield × injury factors] interactions ($\Sigma c_j Ya$) significantly contribute to the description of variation in actual yield Y, indicating that some injuries (or their combinations) had a stronger, or weaker, yield-reducing effect, depending on the level of attainable yield Ya. For instance, yield

losses due to sheath blight, weed infestation, and rice tungro disease tend to increase, remain stable, and decrease, respectively, with increasing attainable yields (Savary et al. 2000b) .

Patterns of injury profiles, along with the attainable yield Ya associated with the corresponding (Savary et al. 2000a) production situations, were then used as scenarios where the statistical model can be used to estimate yield losses to diseases, animal pests, and weeds. Back-computations were performed using the principal component regression model and the numerical values of injuries occurring in specified sub-populations of farmers' fields (sub-populations of farmers' field falling within given [injury profile x production situation] combinations) from the total population of farmers' fields surveyed in Asia (Savary et al. 2000a).

The main results of yield loss estimates using this statistical model are the following:

- For an individual disease, animal pest or weed, sheath blight, brown spot, and leaf blast are diseases that cause important losses (between 1 and 10%) regionally. Among the insect injuries, only white heads caused by stem borers appear of relevance (2.3% yield losses). These injuries, however, do not match in importance those caused by weeds, whether outgrowing the rice crop canopy (WA) or not (WB), both types of injuries causing about 20% yield losses when considered individually.
- For [production situations × injury profiles] combinations across Asia, depending on the scenario, losses ranging from 24 to 41% were found.
- For Asia overall, a mean yield loss of 37.2% was estimated for the combined overall injuries to diseases, animal pests, and weeds. This estimate is derived from the combination of all mean injuries into one mean injury profile occurring regionally, and with a mean regional attainable yield of 5.5 t ha^{-1}. Importantly, this figure indicates that injuries are less than additive in their yield-reducing effects.

Epidemiological Modelling: Some Results

Simulations with EPIRICE lead to distinctive patterns of simulated disease epidemics, which differ between the five diseases with respect to epidemic onset, shape (exponential, sigmoid, sigmoid followed by a decline), and speed (Savary et al., 2012). These patterns generally correspond to those of observed disease epidemics:

- Brown spot: the simulated epidemic is delayed in comparison with the observed data; however it is very fast, with an exponential increase. The observed epidemic has approximately the same shape as the simulated one.
- Leaf blast: the simulated and observed epidemics have very similar shapes, with an early onset and a distinct decline.
- Bacterial leaf blight: the simulated and observed epidemics have similar shapes, with an overall sigmoid pattern ending with a progressive decline.

- Sheath blight: the simulated and observed epidemics have very similar, sigmoid shapes.
- Rice tungro disease: the shape of observed epidemics is similar to the simulated one. However, the observed maximum intensity is higher (50% incidence) than the simulated one (30%).

Simulated global maps of potential rice disease epidemics were produced for the years 1997 to 2008 (Savary et al. 2012):

- Brown spot: The mapped area under disease progress curves (AUDPC, %.day) of brown spot severity ranged from 0 to 902%.day, corresponding to potential epidemics with terminal disease severities of 0–42% (the equivalent of continuous 7.5% from crop establishment to harvest). The largest values (122–902%.day) were predicted in tropical South America, tropical western Africa, and some areas of South and Southeast Asia. The associated standard deviations mostly corresponded to variation in means. However, in some areas, a negative mean-variance relationship was found, as for example in the eastern part of India (large means, low variance) vs. the western part of India and Pakistan (low means, large variance).
- Leaf blast: The maximum range of the simulated potential AUDPC for leaf blast severity, 58–361%.day, corresponded to a maximum severity of 1.6–9.7%. Areas for high average disease were mapped in South America (NE and SE Brazil), Africa (e.g., Madagascar, West Africa, and the Ethiopian highlands), South and Southeast Asia (e.g., the Himalayan foothills, the northern part of the Indo-China peninsula, southern China), and East Asia (e.g., Korea, Japan). Standard deviations were large in areas with large means, but also in some areas with intermediate to average means (e.g., Central North-America and North-East China).
- Bacterial blight: The highest range of AUDPC for bacterial blight leaf incidence was 1681–2677%.day corresponding to a maximum incidence (at the leaf scale) range of 32–52%. Such areas were mapped in the northern half of South America, North and East coasts of the African Gulf of Guinea, East India, the Indo-China peninsula, Eastern China, and Southeast Asian islands. The highest standard deviations were found in Southern Brazil, the Sahel, West India, and patches across East and Southeast Asia.
- Sheath blight: Simulated potential sheath blight epidemics had spatial patterns similar to bacterial blight, except that high ranges of SHB were also found in southern Africa and in Madagascar. These highest ranges, 3783 to 4282%.day, corresponded to maximum incidences of 77–87% (% tillers infected). The largest standard deviations were simulated in, e.g., North America, South America, southern Africa, the Sahel, and North-West India.
- Rice tungro disease: potential epidemics of tungro were simulated in areas where more than one crop of rice is grown per year, i.e., South and Southeast Asia, Madagascar, in limited areas of Africa, and in Guyana and Surinam. The highest potential epidemics were simulated in North-East India, Bangladesh, the Indochina peninsula, South and East China, and patches in the Philippines. The

maximum incidence (% of diseased plants) was 22.7–24.0%, with generally high standard deviations.

There was wide variation between, on the one hand, diseases, and on the other hand locations in the world, in terms of variance-to-mean ratios. Low variance-to-mean ratios indicate endemicity – contexts where disease is always present; high variance-to-mean ratios indicate high variability over time in the occurrence and intensity of epidemics. The analysis of variance-to-mean ratio may lead to characterising contexts of chronic epidemics, as opposed to acute or emerging epidemics. The three types of epidemics call for completely different disease management strategies.

Yield Loss Modelling: Some Results

Simulation modelling with RICEPEST (Willocquet et al. 2004) provides the flexibility required to address varying production situations and diverse pest profiles – this question is relevant for rice health, but also for any crop that is cultivated in a range of contexts. Because it allows thinking in terms of yield gains, instead of yield losses, simulation modelling also enables the implementation (operationalisation; Zadoks 1972) of concepts which pertain to disease management: the management efficacy (i.e., the potential that a given disease management tool has to achieve injury reduction) and the management efficiency (i.e., the potential of a given management tool to generate yield gains) were estimated. A simulation approach therefore allows the modelling of scenarios which explicitly include (i) different pest management strategies, (ii) within the agroecological/technical/economical contexts of rice production (i.e., production situations), and (iii) the multiple injury profiles that are associated with these scenarios.

On the basis of simulated yield losses and of simulated management efficiencies, rice pathogens and pests can be classified into two broad categories of research priority-setting (Willocquet et al. 2004). One group, including weeds, sheath blight, and brown spot, consists of yield-reducing agents for which effective pest management tools need to be developed: the (actual and potential) losses are very high, and the management efficiency is insufficient. The second group consists of leaf blast, neck blast, bacterial leaf blight, and brown planthoppers, for which the efficiency of current management methods is to be maintained: the (actual and potential) losses are very high, the existing management tools – essentially: host-plant resistance genes incorporated in high-yielding rice varieties – are effective, but these tools are vulnerable (resistance genes can be overcome).

The future, with pre-set definition of combined production situations and technologies, can be explored with RICEPEST. Simulated yield losses in future production situations indicated that a new type of rice plant with high harvest index and high biomass production (the “New Plant Type”; Peng et al. 1999; Yang et al. 2007) was more vulnerable to injuries than hybrid rice. Vulnerability refers here to the

potential of harmful organisms, if established, to cause yield reduction -- and so differs from susceptibility, referring to the potential for establishment (Butt and Royle 1980).

Much research has considered the possibility, or the potential, of incorporating resistance genes to stem borers through genetic engineering (High et al. 2004). Our simulations also indicated that the impact of deployment of host resistance (through genetic engineering) was much larger if targeted against sheath blight, rather than against stem borers.

Scenario analyses, encompassing the entire area of Asia covered by farmers' field surveys (Savary et al. 2000b), were also undertaken. Simulated yield losses for combinations of production situations and injury profiles that dominate current lowland rice production in tropical Asia ranged from 140 to 230 g m^{-2} (1.4–2.3 tons. ha^{-1}). For these combinations, the simulated efficiency of current pest management methods, expressed in terms of relative yield gains, ranged from 0.38 to 0.74. Overall, the analyses indicated that 120 to 200 × 10^6 tons of grain yield are lost yearly to pests over the 87 × 10^6 ha of lowland rice in tropical Asia. This also amounts to the potential gain that future pest management strategies could achieve, if deployed.

Some Directions for Future Research

This chapter is not meant to elaborate on the several directions research may take to address plant diseases in rice. One of them, however, is based on the elements outlined earlier, considering epidemiology and yield loss together to provide a view on possible futures. The work of Duku et al. (2016) provides an example of what combined epidemiological and yield-loss modelling can offer, to explore the possible impacts of climate change on rice health. Many such applications are likely in the future.

Recent Trends in Rice Crop Health in Asia

Rice health in the Post-Green Revolution (Savary et al. 2014) has been influenced by simultaneous changes:

1. the deployment of hybrids, along with conventional (inbred) high-yielding varieties,
2. the privatisation of the agricultural supply systems, including an important fraction of breeding programmes,
3. a steady decline of centralised agricultural support systems, especially where, in Asia, they had been extremely strong (e.g., China, Vietnam, Thailand),

4. the steady and continued decline in public investments, especially in agricultural infrastructures (notably, Asia's irrigation systems, which had been the backbone of agricultural development),
5. the sustained, increased demand of a growing Asian population (irrespective of a slow shift in food preference in fractions of the population), along with
6. a progressive rise of prices for commodities accompanied by very high price volatility (see, e.g., the 2008 crisis), and lastly
7. the early signs of climate change, along with pollution impacts (Savary et al. 2014) .

Surveys across Asia indicate a stable level of sheath blight, now established as a major rice disease across the region; a slight overall decline of neck blast; but a distinctive increase in false smut (Savary et al. 2014) . The few sections below provide some additional highlights.

While bacterial blight was a major rice disease in Asia (Ou 1985), no severe epidemics occurred recently because of the cultivation of varieties with resistance genes, despite shifts in the pathogen population (Mew and Vera Cruz 1988, Mew et al. 1992). Disease intensity started to increase in several rice-growing areas in Asia with the widespread cultivation of hybrid rice whose susceptibility was attributed to the narrow genetic base of the parental lines released during this period (Mew and Vera Cruz 1988) and associated cropping practices such as the application of high amounts of nitrogen fertiliser. Bacterial blight is now considered one of the most important diseases of rice in China (McBeath and McBeath 2010). Surveys conducted by the International Rice Research Institute and its partners in the late 2000s showed a high intensity of bacterial blight on very popular varieties, such as Samba Mahsuri (BPT5204) in India and Thien Uu 8 in northern Vietnam, and the increased prevalence of bacterial blight in the Philippines and southern Vietnam.

Sporadic epidemics of blast have been recorded since ancient times (Ou 1985). Results of breeding trials for resistance to blast, which started in the 1960s (Ou 1985), show that the pathogen overcomes resistance shortly after cultivation of newly released varieties. Continued breeding efforts using race-specific resistance genes and quantitative trait loci that confer partial resistance have prevented large-scale epidemics in major rice-growing areas in Asia. However, recent surveys in farmers' fields indicate increased levels of leaf neck blast in Asia in the 2000s compared with those in the 1990s (Hossain et al. 2017; ICAR-IIRR 2018; Savary et al. 2014).

False smut has become a major disease in China where it occurred in approximately 2.4 million hectares of rice annually from 2015 to 2017 (Qiu et al. 2019). The increased frequency in China is associated with cultivation of hybrid rice (Liang et al. 2014; Huang et al. 2019, Wang et al. 2019). In recent years, the prevalence of false smut has also increased in India (Barnwal et al. 2009, Reddy et al. 2011) and Bangladesh (Sarker et al. 2016).

Rice stripe virus disease (RSVD), transmitted by the small brown plant hopper (*Laodelphax striatellus*) showed increased levels in the 1980s in eastern Japan, and were controlled by the deployment of resistant varieties (Iizuka 1989).

Populations of small brown plant hoppers have been increasing since 2000 in some regions in China and Japan (Otuka et al. 2010), leading to severe epidemics, e.g., in Jiangsu, and to expansion to neighbouring provinces, associated with the use of susceptible rice varieties, changes in crop establishment date, and intensive pesticide use Otuka 2013).

The small brown plant hopper (*L. striatellus*) also transmits rice black-streaked dwarf virus (RBSDV). Rice black-streaked dwarf virus disease (RBSDVD) was first observed in Zhejiang in 1989 and severe epidemics were reported in the 1990s, particularly in areas where japonica rice was grown (Wang et al. 2009), spreading later to neighbouring provinces of China as well as South Korea (Lee et al. 2005, Otuka 2013) and Vietnam.

Southern rice black-streaked dwarf virus disease, which is transmitted by the whitebacked plant hopper (*Sogatella furcifera*) , was discovered in southern China in 2001 (Zhou et al. 2013). The disease spread to northern (Zhou et al. 2013) and central Vietnam in 2009 (Hoang et al. 2011), to Japan in 2010 (Matsukura et al. 2013) and Korea in 2016 (Lee et al. 2017). Hybrid rice is generally more susceptible to whitebacked brown plant hopper than japonica (Cheng 2015) or inbred rice (Zhou et al. 2013). The population of plant hoppers in China was found to be higher on early to late summer rice than on spring rice (Zhou et al. 2013).

Red stripe is an emerging rice disease, first reported in Indonesia (Mogi et al. 1988), and observed since in several countries in Southeast Asia (Barroga and Mew 1994; Pham et al. 1991), and India (Krishnam Raju et al. 2012).

Severe infestations by the brown plant hopper, *Nilaparvata lugens*, have been reported in recent years. Outbreaks were recorded in the Yangtze River Delta in 2005 (Hu et al. 2014), in Vietnam (2006–2007; Catindig et al. 2009), and in the central plains of Thailand (2008–2012; Escalada et al. 2015). The brown plant hopper is a vector for the ragged stunt and grassy stunt viral disease; co-infection by both viruses causes a yellowing syndrome, first observed in southern Vietnam in 1989 (Pham et al. 2005), with severe epidemics reported in 2006 to 2008 (Cabauatan et al. 2009).

Conclusion: Some Perspectives

Research on rice health in the past 30 years has completely changed our view on the importance of pathogens, animal pests, and weeds, and on the nature of the problems we face in managing plant health. Some of the main elements may be listed as follows:

- Plant pathogens are indeed very important yield reducers in rice. Compared to what was envisioned in the 1980s, plant diseases appear to be much more important reducing factors than insects.
- Plant pathogens also can gravely affect the nutritional quality of rice grains. This aspect has been only marginally addressed in this chapter. Several rice pathogens

are to be considered in association with this issue. Mycotoxin accumulation is a serious concern, which should be fully addressed.

- The assumed importance of insect pests in the 1980s may be due to experimental and analytical biases in the way insect injuries and losses were studied, analysed, and quantified – this may have coincided with the expansion of pesticide use in Asia, even before the implementation of the Green Revolution.
- But it also is possible that, because of tremendous change in plant materials, resulting from the massive breeding programmes of the Green Revolution and the wide diffusion of improved materials to national breeding programmes, the cultivated rice varieties have become much less susceptible to a range of insects. This hypothesis, to our knowledge, has never has been seriously explored.
- Nevertheless, and irrespective of the rice production situation considered, weed infestation is the main cause for chronic losses.
- There is a constant, continuous change in rice production situations in Asia. The respective importance of plant pathogens also evolves rapidly.
- While the levels of chronic losses to diseases are high, the risk of very severe -- much higher -- rice yield losses in Asia, caused by emerging or acute plant diseases, is very real.
- Because of the role of rice in Asian nutrition -- irrespective of current dietary trends -- and because of the shape of the international market, a major epidemic in one of the "rice bowls" of Asia could have grave local and regional consequences.
- Science is required to identify disease risks, to characterise the epidemiological threats to plant health, and to design disease management strategies.

References

Agresti, A. (2002). *Categorial data analysis* (2nd ed.). Hoboken: Wiley.

Ahrens, C., Cramer, H. H., Mogk, M., & Peschel, H. (1982). Economic impact of crop losses. In *Proceedings 10th International Congress of Plant Protection* (pp. 65–73). Farnham: British Crop Protection Council.

Alam, M. Z. (1961). *Insect pests in East Pakistan and their control*. Dacca: East Pakistan Department of Agriculture.

Alam, M. Z. (1967). Insect pest of rice in East Pakistan. Pages 643–655. In *The major insect pests of the rice plant. Proceedings of a symposium at the International Rice Research Institute. September, 1964*. Baltimore: The Johns Hopkins Press.

Alam, M. Z., Alam, M. S., & Abbas, M. (1972). Status of different stem borers as pests of rice in Bangladesh. *International Rice Commission Newsletter, 21*(2), 279.

Allen, T. H. F., & Starr, T. B. (1982). *Hierarchy – Perspectives for ecological complexity*. Chicago/ London: The University of Chicago Press.

Azzam, O., & Chancellor, T. C. B. (2002). The biology, epidemiology, and management of rice tungro disease in Asia. *Plant Disease, 86*, 88–100.

Barnwal, M. K., Agarwal, B. K., Sathi, S. K., & Mahato, D. (2009). Effect of different nitrogen sources on false smut disease and yields in commercial rice hybrids. *Journal of Plant Protection and Environment, 6*, 95–98.

Barr, B. A., Koehler, C. S., & Smith, R. F. (1975). Crop losses - rice: field losses to insects, diseases, weeds, and other pests. UC/AID Pest Management and Related Environmental Protection Project. University of California, Berkeley, 64 pp.

Barroga, J. F., & Mew, T. W. (1994). *"Red stripe": A new disease of rice in the Philippines*. In Paper presented at the 25th anniversary and annual workshop on rice disease management technology in the tropics, scientific convention of the Pest Management Council of the Philippines, Cagayan de Oro City, Philippines, 3–6 May 1994.

Benzécri, J. P. (1973a). *L'analyse des données*, Vol. 1. La Taxinomie. Paris: Dunod.

Benzécri, J. P. (1973b). *L'analyse des données*, Vol. 2. L'Analyse des Correspondances. Paris: Dunod.

Breslow, L. (1978). Risk factor intervention for health maintenance. *Science, 200*, 908–912.

Butt, D. J., & Royle, D. J. (1980). The importance of terms and definitions for a conceptually unified epidemiology. In J. Palti & J. Kranz (Eds.), *Comparative epidemiology – A tool for better disease management* (pp. 29–45). Wageningen: Pudoc.

Cabauatan, P. Q., Cabunagan, R. C., & Choi, I. R. (2009). Rice viruses transmitted by the brown planthopper *Nilaparvata lugens* Stål. In K. L. Heong & B. Hardy (Eds.), *New threats to the sustainability of intensive rice production systems in Asia* (pp. 357–368). Los Baños: International Rice Research Institute.

Castilla, N. P., Leaño, R. M., Elazegui, F. A., Teng, P. S., & Savary, S. (1996). Effects of plant contacts, inoculation pattern, leaf wetness regime, and nitrogen supply on inoculum efficiency in rice sheath blight. *Journal of Phytopathology, 144*, 187–192.

Catindig, J. L. A., Arida, G. S., Baehaki, S. E., Bentur, J. S., Cuong, J. S., Norowi, M., Rattanakarn, W., Sriratanasak, W., Xia, J., & Lu, Z. (2009). Situation of planthoppers in Asia. In K. L. Heong & B. Hardy (Eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia* (pp. 191–220). Los Baños: International Rice Research Institute.

Chakrabarti, N. K. (2001). Epidemiology and disease management of brown spot of rice in India. In S. Sreenivasaprasad & R. Johnson (Eds.), *Major diseases of rice – Recent advances* (pp. 293–306). Dordrecht: Kluwer Academic Publishers.

Chauhan, L. S., Verman, S. C., & Rajpai, G. K. (1968). Assessment of losses due to stem rot of rice caused by Sclerotium oryzae. *Plant Disease Report, 52*, 963–965.

Cheng, J. (2015). Rice planthoppers in the past half century in China. In K. L. Heong, J. Cheng, & M. M. Escalada (Eds.), *Rice planthoppers: Ecology, management, socio economics and policy* (pp. 1–33). Hangzhou/Dordrecht: Zhejiang University Press/Springer.

Chiarappa, L. (Ed.). (1971). *Crop loss assessment methods: FAO manual on the evaluation and prevention of losses by pests, diseases and weeds*. Farnham Royal: Commonwealth Agricultural Bureaux.

Church, B. M., & Austin, R. B. (1983). Variability of wheat yields in England and Wales. *The Journal of Agricultural Science, 100*, 201–204.

Cramer, H. H. (1967). Plant protection and world crop production. *Pflanzenschutz Nachr. Bayer, 20*, 1–524.

Dasgupta, M. K., & Chattopadhyay, S. B. (1977). Effect of environmental factors on the development of brown spot of rice caused by *Helminthosporium oryzae*. *Journal of Society Experimental Agriculture, 2*, 24–27.

De Wit, C. T., & Penning de Vries, F. W. T. (1982). L'analyse des systèmes de production primaires. In F. W. T. Penning de Vries & M. A. Djiteye (Eds.), *La productivité des pâturages Sahéliens* (Agriculture Research Report, 918) (pp. 275–283). Wageningen: Pudoc.

Draper, N. R., & Smith, H. (1981). *Applied regression analysis* (2nd ed.). New York: Wiley.

Duku, C., Sparks, A. H., & Zwart, S. J. (2016). Spatial modelling of rice yield losses in Tanzania due to bacterial leaf blight and leaf blast in a changing climate. *Climatic Change, 135*, 569–583.

Elazegui, F. A., Soriano, J., Bandong, J., Estorninos, L., Jonson, I., Teng, P. S., Shepard, B. M., Litsinger, J. A., Moody, K., & Hibino, H. (1990). Methodology used in the IRRI integrated pest survey. In *Crop loss assessment in rice* (pp. 243–271). Los Baños: International Rice Research Institute.

El-Refaei, M. I. (1977). *Epidemiology of rice blast disease in the tropics with special reference to the leaf wetness in relation to disease development. PhD thesis*. New Delhi: Indian Agricultural Research Institute.

Escalada, M. M., Luecha, M., & Heong, K. L. (2015). Social impacts of planthopper outbreaks: Case study from Thailand. In K. L. Heong, J. Cheng, & M. M. Escalada (Eds.), *Rice planthoppers: Ecology, management, socio economics and policy* (pp. 209–226). Hangzhou/Dordrecht: Zhejiang University Press/Springer.

Fernando, H. E. (1966). Recent development in paddy pest control. In D. V. W. Abeygunawardena (Ed.), *Proceedings of a symposium on research and production of rice in Ceylon* (pp. 120–130). Colombo: Ceylon Association for the Advancement of Science.

Forbes, G. A., & Jeger, M. J. (1987). Factors affecting the estimation of disease intensity in simulated plant structures. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 94*, 113–120.

Ghose, R. L. M., Ghatge, M. B., & Subrahmanyan, V. (1960). *Rice in India* (Rev. ed). Indian Council of Agricultural Research: New Delhi, 474 p.

Greenacre, M. J. (1984). *Theory and applications of correspondence analysis*. London: Academic.

Greenland, D. J. (1997). *The sustainability of rice farming*. Wallingford/Los Baños: CAB International/International Rice Research Institute.

Harrell, F. E., Jr. (2001). *Regression modeling strategies: With applications to linear models, logistic regression, and survival analysis*. New York: Springer.

Heong, K. L., & Ho, N. K. (1987). Farmers' perceptions of the rice tungro problem in the Muda irrigation scheme, Malaysia. Pages 165–174. In J. Tait, D. Bottrell, & B. Napompeth (Eds.), *Management of pests and pesticides: Farmers' perceptions and practices*. Boulder: Westview Press.

Hernandez, A. (1923). Report of the plant disease section. *Report on Plant Disease Section. Philippines Bureau of Agriculture, 23*, 159–172.

High, S. M., Cohen, M. B., Shu, Q. Y., & Altosaar, I. (2004). Achieving successful deployment of Bt rice. *Trends in Plant Science, 9*, 286–292.

Hill, M. O. (1974). Correspondence analysis: A neglected multivariate method. *Applied Statistics, 23*, 340–354.

Hoang, A. T., Zhang, H. M., Yang, J., Chen, J. P., Hébrard, E., Zhou, G. H., Vinh, V. N., & Cheng, J. A. (2011). Identification, characterization, and distribution of southern rice black-streaked dwarf virus in Vietnam. *Plant Disease, 95*, 1063–1069.

Hossain, M., Ali, M. A., & Hossain, M. D. (2017). Occurrence of blast disease in rice in Bangladesh. *American Journal of Agriculture Science, 4*, 74–80.

Hu, G., Lu, F., Zhai, B. P., Lu, M. H., Liu, W. C., Zhu, F., Wu, X. W., Chen, G. H., & Zhang, X. X. (2014). Outbreaks of the brown planthopper *Nilaparvata lugens* (Stål) in the Yangtze River Delta: Immigration or local reproduction? *PLoS One, 9*(2), e88973. https://doi.org/10.1371/journal.pone.0088973.

Huang, S., Liu, L., Wang, L., & Hou, Y. (2019). Research on advance of rice false smut *Ustilaginoidea virens* (Cooke) Takah worldwide: Part I. Research status of rice false smut. *Journal of Agricultural Science, 11*(15), 240. https://doi.org/10.5539/jas.v11n15p240.

Iizuka, K. (1989). Trend of rice stripe virus viruliferous rate in northern Kanto district and pest management. *Agriculture and Horticulture, 64*, 965–970.

Indian Council of Agricultural Research-Indian Institute of Rice Research (ICAR-IIRR). (2018). *Production oriented survey 2017. All India Coordinated Rice Improvement Programme*. Rajendranagar: ICAR-Indian Institute of Rice Research.

Jayaraj, S., Velayutham, B., Rathinasamy, C., & Regupathy, A. (1974). Damage potential of the brown planthopper on rice. *Madras Agriculture Journal, 61*, 144–148.

Jeger, M. J. (2000). Bottlenecks in IPM. *Crop Protection, 19*(8–10), 787–792.

Kauffman, H. E., & Kannaiyan, S. (1987). Bacterial blight of rice. In S. Kannaiyan (Ed.), *Advances in rice pathology* (pp. 265–292). Coimbatore: Tamil Nadu Agricultural University.

Krishnam Raju, S., Bhuvaneswari, V., & Madhusudhan, P. (2012). Quantification of yield loss caused by red stripe disease in rice. *Oryza, 49*, 313–315.

Lee, B. C., Hong, Y. K., Hong, S. J., Park, S. T., & Lee, K. W. (2005). Occurrence and detection of rice black-streaked dwarf virus in Korea. *Plant Pathology Journal, 21*, 172–173.

Lee, B. C., Bae, J. Y., Kim, S. M., Ra, J. E., Choi, N. J., Choi, M. Y., & Park, K. D. (2017). *First report of southern rice black streaked dwarf virus in South Korea and simultaneous detection of RBSDV and SRBSDV*. In: Proceedings of the Korean Society of Crop Science Conference Korean Society of Crop Science Conference, p. 24.

Liang, Y., Zhang, X., Li, D., Huang, F., Hu, P., & Peng, Y. (2014). Integrated approach to control false smut in hybrid rice in Sichuan province, China. *Rice Science, 21*, 354–360.

Litsinger, J. A., Canapi, B. L., Bandong, J. P., Cruz, C. D., Apostol, R. F., Pantua, P. C., Lumaban, M. D., Alviola, A. L., Raymundo, F., Libetario, E. M., & Loevinsohn, M. E. (1987). Rice crop loss from insect pests in wetland and dryland environments of Asia with emphasis on the Philippines. *International Journal of Tropical Insect Science, 8*, 677–692.

Matsukura, K., Towata, T., Sakai, J., Onuki, M., Okuda, M., & Matsumura, M. (2013). Dynamics of southern rice black-streaked dwarf virus in rice and implication for virus acquisition. *Phytopathology, 103*, 509–512.

McBeath, J. H., & McBeath, J. (2010). *Environmental change and food security in China*. Dordrecht: Springer.

Mew, T. W. (1987). Current status and further prospects of research on bacterial blight of rice. *Annual Review of Phytopathology, 25*, 359–382.

Mew, T. W., & Vera Cruz, C. M. (1988). Background resistance to bacterial blight (BB) hill and leaf infection. *International Rice Research Newsletter, 13*(2), 11–12.

Mew, T. W., Vera Cruz, C. M., & Medalla, E. S. (1992). Changes in the race frequency of *Xanthomonas oryzae* pv. oryzae in response to rice cultivars planted in the Philippines. *Plant Disease, 76*, 1029–1032.

Mogi, S., Sugandhi, Z., & Baskoro, S. (1988). *A new discovered disease (bacterial red stripe) on rice at Indonesia, its symptoms and distribution*. In Abstracts of the 5th international congress of plant pathology, International Conference Hall, Kyoto, Japan, 20–27 August 1988.

Monteith, J. L. (1977). Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society B, 281*, 277–294.

Moody, K. (1982). The status of weed control of rice in Asia. *FAO Plant Protection Bulletin, 30*, 119–123.

Nuque, F. L. (1963). *Panicle blast outbreak in Leyte Province. Report to the IRRI Director General*, 3p.

Nuque, F. L. (1970). *Panicle blast outbreak in Laguna and Quezon Provinces. Report to the IRRI Director General*, 2p.

Otuka, A. (2013). Migration of rice planthoppers and their vectored re-emerging and novel rice viruses in East Asia. *Frontiers Microbiology, 28*(4), 309. https://doi.org/10.3389/fmicb.2013.00309.

Otuka, A., Matsumura, M., Sanada-Morimura, S., Takeuchi, H., Watanabe, T., Ohtsu, R., & Inoue, H. (2010). The 2008 overseas mass migration of the small brown planthopper, *Laodelphax striatellus*, and subsequent outbreak of rice stripe disease in western Japan. *Applied Entomology and Zoology, 45*, 259–266.

Ou, S. H. (1985). *Rice diseases* (2nd ed.). Surrey: Commonwealth Mycological Institute.

Ou, S. H., & Bandong, J. M. (1976). Yield losses due to sheath blight of rice. *International Rice Research Newsletter, 1/76*, 14.

Padmanabhan, S. Y. (1965). Estimating losses from rice blast in India. Pages 203–221. In *The rice blast disease. Proceedings of a symposium at the International Rice Research Institute, July 1963*. Baltimore: The Johns Hopkins Press.

Padmanabhan, S. Y. (1973). The great Bengal famine. *Annual Review of Phytopathology, 11*, 11–26.

Pannu, P. P. S., Chahal, S. S., Kaur, M., & Sidhu, S. S. (2005). Influence of weather variables on the development of brown leaf spot caused by *Helminthosporium oryzae* in rice. *Indian Phytopathology, 58*, 489–492.

Pathak, P. K., & Dhaliwal, G. S. (1981). *Trends and strategies for rice pest problems in tropical Asia* (IRRI Research Papers Series 64). Los Banos: IRRI.

Peng, S., Cassman, K. G., Virmani, S. S., Sheehy, J., & Khush, G. S. (1999). Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. *Crop Science, 6*, 1552–1560.

Pham, V. D., Nguyan, T. P. L., & Hoang, D. D. (1991). Red stripe, a newly reported disease of rice in Vietnam. *International Rice Research Newsletter, 16*(3), 25.

Pham, V. D., Cabunagan, R. C., & Choi, I. R. (2005). Rice "yellowing syndrome" in Mekong River Delta. *Omonrice, 13*, 135–138.

Pruthi, H. S. (1953). An epidemic of rice bug in India. *FAO Plant Protection Bulletin, 1*, 87–88.

Qiu, J., Meng, S., Deng, Y., Huang, S., & Kou, Y. (2019). *Ustilaginoidea virens*: A fungus infects rice flower and threats world rice production. *Rice Science, 26*, 199–206.

Reddy, D. B. (1967). The rice gall midge Pachydiplosis oryzae (Wood-Mason). In *The major insect pests of the rice plant. Proceedings of a symposium at the International Rice Research Institute. September, 1964* (pp. 457–491). Baltimore: The Johns Hopkins Press.

Reddy, D. B. (1973). High yielding varieties and special plant protection problems with particular reference to tungro virus of rice. *International Rice Commission Newsletter, 22*(2), 34–42.

Reddy, C. S., Laha, G. S., Prasad, M. S., et al. (2011). Characterizing multiple linkages between individual diseases, crop health syndromes, germplasm deployment, and rice production situations in India. *Field Crops Research, 120*, 241–253.

Sarker, M. M., Haque, A. H. M. M., Nessa, B., Salam, M. U., Islam, M. M., & Muqit, A. (2016). Status of rice false smut disease in Natore District of Bangladesh. *Bangladesh Rice Journal, 20*, 31–37.

Savary, S., Elazegui, F. A., Moody, K., Litsinger, J. A., & Teng, P. S. (1994). Characterization of rice cropping practices and multiple pest systems in the Philippines. *Agricultural Systems, 46*, 385–408.

Savary, S., Elazegui, F. A., & Teng, P. S. (1996). *A survey portfolio for the characterization of rice pest constraints* (IRRI Discussion Paper Series No. 18). Manila: International Rice Research Institute.

Savary, S., Elazegui, F. A., Pinnschmidt, H. O., Castilla, N. P., & Teng, P. S. (1997a). *A new approach to quantify crop losses due to rice pests in varying production situations* (IRRI Discussion Paper Series No. 20). Manila: International Rice Research Institute.

Savary, S., Willocquet, L., & Teng, P. S. (1997b). Modelling sheath blight epidemics on rice tillers. *Agricultural Systems, 55*, 359–384.

Savary, S., Elazegui, F. A., & Teng, P. S. (1998). Assessing the representativeness of data on yield losses due to rice diseases in tropical Asia. *Plant Disease, 82*, 705–709.

Savary, S., Willocquet, L., Elazegui, F. A., Teng, P. S., Du, P. V., Zhu, D., Tang, Q., Huang, S., Lin, X., Singh, H. M., & Srivastava, R. K. (2000a). Rice pest constraints in tropical Asia: Characterization of injury profiles in relation to production situations. *Plant Disease, 84*, 341–356.

Savary, S., Willocquet, L., Elazegui, F. A., Castilla, N. P., & Teng, P. S. (2000b). Rice pest constraints in tropical Asia: Quantification of yield losses due to rice pests in a range of production situations. *Plant Disease, 84*, 357–369.

Savary, S., Teng, P. S., Willocquet, L., & Nutter, F. W. (2006). Quantification and modelling of yield losses. *Annual Review of Phytopathology, 44*, 89–112.

Savary, S., Mila, A., Willocquet, L., Esker, P. D., Carisse, O., & McRoberts, N. (2011). Risk factors for crop health under global change and agricultural shifts: A framework of analyses using rice in tropical and subtropical Asia as a model. *Phytopathology, 101*, 696–709.

Savary, S., Nelson, A., Willocquet, L., Pangga, I., & Aunario, J. (2012). Modeling and mapping potential epidemics of rice diseases globally. *Crop Protection, 34*, 6–17.

Savary, S., Esker, P. D., McRoberts, N., & Willocquet, L. (2014). Environmental interactions and multiple forcing leading to shifts and variability of crop yield losses. In: Abstracts of the APS-CPS joint meeting, Minneapolis, Minnesota, 9–13 August 2014. *Phytopathology, 104*, 150.

Savary, S., McRoberts, N., Esker, P. D., Willocquet, L., & Teng, P. S. (2017). Production situations as drivers of crop health: Evidence and implications. *Plant Pathology, 66*, 867–876.

Shane, W. W., & Teng, P. S. (1987). Generating the data base for disease-loss modelling. In P. S. Teng (Ed.), *Crop loss assessment and pest management* (pp. 82–89). St. Paul: APS Press.

Soenardi, I. (1967). Insect pests of rice in Indonesia. Pages 675–683. In *The major insect pests of the rice plant. Proceedings of a symposium at the International Rice Research Institute. September, 1964*. Baltimore: The Johns Hopkins Press.

Sokal, R. R., & Rohlf, F. J. (1981). *Biometry* (2nd ed.). San Francisco: WH Freeman.

Srivastava, D. N. (1967). Epidemiology and control of bacterial blight of rice in India. Pages 11–18. In *Proceedings of a symposium on rice diseases and their control by growing resistant varieties and other measures*. Tsukuba: Agriculture, Forestry, and Fisheries Research Council.

Steinberg, D., & Colla, P. (2007). Logistic regression. In *Statistics III, SYSTAT 12* (pp. 2–92). San Jose: SYSTAT Software.

Teng, P. S. (1981). Validation of computer models of plant disease epidemics: A review of philosophy and methodology. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 88*, 49–63.

Teng, P. S. (1986). Crop loss appraisal in the tropics. *Journal of Tropical Plant Protection, 3*(1), 39–50.

Teng, P. S. (Ed.). (1987). *Crop loss assessment and pest management*. St. Paul: APS Press.

Teng, P. S. (Ed.). (1990a). *Crop loss assessment in rice*. In Papers given at the International workshop on crop loss assessment to improve pest management in rice and rice-based cropping systems in South and Southeast Asia, Los Baños, Philippines, 11–17 Oct 1987. International Rice Research Institute: Manila.

Teng, P. S. (1990b). *IPM in rice: An analysis of the status quo with recommendations for action. Report to the International IPM Task Force (FAO/ACIAR/IDRC/NRI)*. Los Baños: International Rice Research Institute.

Teng, P. S. (1994a). The epidemiological basis for blast management. In R. S. Zeigler, S. A. Leong, & P. S. Teng (Eds.), *Rice blast disease* (pp. 409–433). Los Baños: CAB International/ Wallingford and International Rice Research Institute.

Teng, P. S. (1994b). Integrated pest management in rice. *Experimental Agriculture, 30*, 115–137.

Teng, P. S., & Gaunt, R. E. (1980). Modelling systems of disease and yield loss in cereals. *Agricultural Systems, 6*, 131–154.

Teng, P. S., Torres, C. Q., Nuque, F. L., & Calvero, S. B. (1990). Current knowledge on crop losses in tropical rice. In *Crop loss assessment in rice. Papers given at the international workshop on crop loss assessment to improve pest management in rice and rice-based cropping systems in South and Southeast Asia, 11–17 Oct 1987, Los Baños, Philippines* (pp. 39–53). Manila: International Rice Research Institute.

Van Ittersum, M. K., & Rabbinge, R. (1997). Concepts in production ecology for analysis and quantification of agricultural input-output combinations. *Field Crops Research, 52*, 197–208.

Vidhyasekaran, P., & Ramados, N. (1973). Quantitative and qualitative losses in paddy due to helminthosporiose epidemic. *Indian Phytopathology, 26*, 479–484.

Wang, H. D., Chen, J. P., Wang, A. G., Jiang, X. H., & Adams, M. J. (2009). Studies on the epidemiology and yield losses from rice black-streaked dwarf disease in a recent epidemic in Zhejiang province, China. *Plant Pathology, 58*, 815–825.

Wang, W. M., Fan, J., & Jeyakumar, J. M. J. (2019). Rice false smut: An increasing threat to grain yield and quality. In Y. Jia (Ed.), *Protecting rice grains in the post-genomic era*. https://doi.org/10.5772/intechopen.84862.

Wathanakul, L., & Weerapat, P. (1969). Virus diseases of rice in Thailand. In *The virus diseases of the rice plant. Proceedings of a symposium at the International Rice Research Institute, April, 1967* (pp. 79–85). Baltimore: The Johns Hopkins Press.

Way, M. J. (1976). Entomology and the world food situation. *Bulletin of the Entomological Society of America, 22*, 125–129.

Wilkinson, L., Engelman, L., Corter, J., & Coward, M. (2007). Cluster analysis. In *SYSTAT 12 statistics I* (pp. 65–124). San Jose: SYSTAT Software, Inc.

Willett, W. C. (2002). Balancing life-style and genomics research for disease prevention. *Science, 296*, 695–698.

Willocquet, L., Savary, S., Fernandez, L., Elazegui, F., & Teng, P. S. (1998). *Simulation of yield losses caused by rice diseases, insects, and weeds in tropical Asia* (IRRI Discussion Paper Series No. 34). Los Baños: International Rice Research Institute.

Willocquet, L., Savary, S., Fernandez, L., Elazegui, F., & Teng, P. S. (2000). Development and evaluation of a multiple-pest, production situation specific model to simulate yield losses of rice in tropical Asia. *Ecological Modelling, 131*, 133–159.

Willocquet, L., Savary, S., Fernandez, L., Elazegui, F. A., Castilla, N., Zhu, D., Tang, Q., Huang, S., Lin, X., Singh, H. M., & Srivastava, R. K. (2002). Structure and validation of RICEPEST, a production situation-driven, crop growth model simulating rice yield response to multiple pest injuries for tropical Asia. *Ecological Modelling, 153*, 247–268.

Willocquet, L., Elazegui, F. A., Castilla, N., Fernandez, L., Fischer, K. S., Peng, S., Teng, P. S., Srivastava, R. K., Singh, H. M., Zhu, D., & Savary, S. (2004). Research priorities for rice pest management in tropical Asia: A simulation analysis of yield losses and management efficiencies. *Phytopathology, 94*, 672–682.

Wyatt, I. J. (1957). *Field investigations of padi stem-borers, 1955–1956*. Bulletin No. 102. Federation of Malaya Department of Agriculture, 42p.

Yang, W., Peng, S., Laza, R. C., Visperas, R. M., & Dionisio-Sese, M. L. (2007). Grain yield and yield attributes of new plant type and hybrid rice. *Crop Science, 47*, 1393–1400.

Zadoks, J. C. (1971). Systems analysis and the dynamics of epidemics. *Phytopathology, 61*, 600–610.

Zadoks, J. C. (1972). Methodology of epidemiological research. *Annual Review of Phytopathology, 10*, 253–276.

Zadoks, J. C. (1985). On the conceptual basis of crop loss assessment: The threshold theory. *Annual Review of Phytopathology, 23*, 455–473.

Zadoks, J. C., & Schein, R. D. (1979). *Epidemiology and plant disease management*. New York: Oxford University Press.

Zeigler, R. S., & Savary, S. (2009). Plant diseases and the world's dependence on rice. In *The role of plant pathology in food safety and food security* (pp. 3–9). Dordrecht: Springer.

Zhou, G., Xu, D., Xu, D., & Zhang, M. (2013). Southern rice black-streaked dwarf virus: A white-backed planthopper-transmitted fijivirus threatening rice production in Asia. *Frontiers Microbiology, 4*, relative270. https://doi.org/10.3389/fmicb.2013.00270.

Chapter 7
Importance of *Fusarium* spp. in Wheat to Food Security: A Global Perspective

Toni Petronaitis, Steven Simpfendorfer, and Daniel Hüberli

Abstract *Fusarium* species are responsible for causing two major diseases in wheat worldwide: *Fusarium* head blight (FHB) and *Fusarium* crown rot (FCR). These diseases threaten wheat production worldwide and have the potential to impact food security negatively, especially since it is estimated that global wheat production will need to increase by around 60% over the next thirty years to meet the demands of the increasing population. FHB and FCR epidemics have become more frequent and widespread in recent times, with pressures from climate change, conservation agriculture and the increasing adoption of wheat-maize rotations contributing to this trend. This review provides a synopsis of yield loss in wheat from *Fusarium* species at a global level, covering briefly each major wheat-producing region, and discusses the impacts of these losses from a perspective of food security. Asian regions, particularly China and India, stand to benefit the most from reducing yield losses to *Fusarium* species as they produce the most wheat for domestic consumption, have the largest population and are vulnerable to food security shocks (e.g. losses of production due to disease). This is amplified by the increasing incidence of FCR in these countries in recent seasons. Although significant research efforts have been made to control FHB and FCR, e.g. crop breeding and integrated disease management (IDM), the pressure on cropping systems to meet the cereal requirements of a growing population, along with climate change and social/political pressures (e.g. plant-based fuel production, political unrest), will bring new challenges.

T. Petronaitis (✉) · S. Simpfendorfer
NSW Department of Primary Industries, Tamworth, NSW, Australia
e-mail: toni.petronaitis@dpi.nsw.gov.au; steven.simpfendorfer@dpi.nsw.gov.au

D. Hüberli
Department of Primary Industries and Regional Development, Perth, WA, Australia
e-mail: daniel.huberli@dpird.wa.gov.au

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_7

Introduction

Global wheat production is currently around 749.5 million tonnes (Megatonnes, Mt) per year. It is predicted that this will need to rise by 60% (up to 1200 Mt) by 2050 to meet the demands of an increasing global population (FAO et al. 2017). Plant pests and pathogens are a serious threat to global food security (Strange and Scott 2005). *Fusarium* species are major pathogens of cereal crops including wheat, maize, barley, rye, oats and rice worldwide, causing both chronic infections and devastating epidemics. *Fusarium* species cause two main diseases, *Fusarium* head blight (scab) (FHB) and *Fusarium* crown (foot) rot (FCR), of bread wheat (*Triticum aestivum*) and durum wheat (*T. turgidum*) (Strange and Scott 2005). These diseases have a significant impact on production (yield), and the mycotoxins produced by *Fusarium* infection have implications for food safety (Proctor et al. 1995; Tanaka et al. 1988).

The United Nations 2030 Agenda for Sustainable Development aims to "end hunger, achieve food security, improve nutrition and promote sustainable agriculture" (FAO et al. 2017). Currently there are 31 countries in Africa, seven countries in Asia and one country in South America identified by FAO (June 2018) to be in food "crisis" (i.e. requiring external assistance). The number of undernourished people is estimated to have increased in 2016 to 815 million (up from 777 million in 2015). Conflicts, along with climate-related shock, such as extreme weather events, were identified as significant contributing factors behind this recent increase (FAO et al. 2017). Considering global food security has been decreasing since 2014, this appears to be an already ambitious and potentially impossible goal to reach within the next decade.

The global population is estimated to reach 9.8 billion by 2050, an increase of more than 2 billion compared with 2017 (UN DESA 2017). To meet the nutritional needs of this future global population, it is estimated that food production needs to increase by at least 50% worldwide by 2050, and wheat production alone by 60% (FAO et al. 2017). Godfray et al. (2010) predicted that a 70–100% increase in agricultural output is required, taking into consideration the challenges of energy and water security, food affordability, climate change and conservation of land. On top of this, there is the risk of increased crop intensification having a negative effect on the yield of important food crops. This is concerning, particularly when yield-limiting factors such as diseases, weeds and pests already have significant effects on food production (Oerke et al. 2012).

Cereals including wheat, rice and maize contribute approximately 50% of the calorie intake of humans. Of these, wheat provides the most nourishment in terms of calories and protein, being the first source of protein and second source of calories (after rice) in diets in most developing countries. Wheat is grown on all continents except Antarctica (FAO 2018), and on a greater land area than any other crop (220 million ha in 2016). The major wheat-producing countries in 2016 included China, India, Russia, the USA and Canada (Table 7.1). Worldwide production of wheat in 2016 was estimated at 749.5 Mt (FAO 2018). Wheat is the dominant staple in temperate zones such as West Asia and North Africa (Turkey, Egypt) and

Table 7.1 Top ten wheat producing countries in 2016, their total wheat production (Mt), the percentage contributed to the total amount of wheat produced worldwide (% of 749.5 Mt), the amount of wheat exported (Mt) and the percentage contributed to world export supply (%)

Country	Production (Mt)	Proportion of world supply (%)	Export supply (Mt)	Proportion of world exports (%)
China	131.7	17.6	0.01	0.0
India	93.5	12.5	0.2	0.1
Russia	73.3	9.8	25.3	13.8
USA	62.9	8.4	24.0	13.1
Canada	30.5	4.1	19.7	10.7
France	29.5	3.9	18.3	10.0
Ukraine	26.1	3.5	11.7	6.4
Pakistan	26.0	3.5	0.0	0.0
Germany	24.5	3.3	10.2	5.5
Australia	22.3	3.0	16.1	8.8
Other[a]	229.2	30.4	58.0	31.6

Data are estimates from FAO (retrieved on 09/19/2018 from http://www.fao.org/faostat/en/#data/QC)

[a]Other countries include Turkey, Argentina, Kazakhstan, United Kingdom, Iran, Poland, Egypt, Romania and Italy

Sub-Saharan Africa (Nigeria, South Africa), and consumption is increasing in Central Asia (China, India) (Shewry and Hey 2015). Given the importance of wheat in the human diet as a staple food, and through its use as a feed for meat production, any loss in wheat production would have an immediate effect on food availability (Nellemann et al. 2009).

Fungal and bacterial diseases are estimated to be responsible for total annual losses in wheat of approximately 12.4%, followed closely by weeds at 12.3% and animal pests at 9.3% (Oerke et al. 2012). These losses vary considerably for different regions. For example, Russia and Australia have the highest losses to disease of all major wheat-producing regions, both having estimated annual yield losses of approximately 17%, or approximately 27.6 Mt for Russia and 4.1 Mt for Australia (Oerke et al. 2012). Although estimated yield loss to diseases is lower in Asia (12%), this is still equivalent to approximately 33.6 Mt of wheat each year. These losses have a more immediate impact on food security at a local scale due to the large population sizes and vulnerability of some Asian countries to food insecurity; currently six countries (Yemen, Afghanistan, Syria, Iraq, Palestine and Bangladesh) are in food crisis (FSIN 2018). The food crisis in many of these countries is intensified by ongoing political unrest.

All wheat-growing regions experience production losses from *Fusarium* species, which are ubiquitous pathogens in agricultural systems worldwide. Epidemics of FHB and FCR are sporadic as they are influenced heavily by the climatic conditions that prevail at specific host crop growth stages. Inoculum primarily survives between wheat crops in cereal and/or grass weed residues. Hence, there is a higher incidence and severity of infection in low- and no-tillage stubble-retention farming systems for both FHB (Dill-Macky and Jones 2000) and FCR (Alahmad et al. 2018).

The major impacts of FHB and FCR on wheat include yield and quality losses, grain availability and mycotoxin contamination, which threaten food and feed safety and decrease grain market value. Yield losses from FHB in small grains were reviewed by Parry et al. (1995) based on approximated historical data following severe epidemics in the period from 1919 to the mid-1990s. These estimates ranged from 15–29% in India to 30–40% in the Yangtze Valley in China, and up to 70% in some areas of Romania (Parry et al. 1995). In the USA, FHB is the most important disease in wheat, responsible for severe epidemics in which yield losses exceeded 30% in individual fields (Bockus et al. 2010). Further economic losses are experienced through reductions in the value of grain due to mycotoxin contamination. In some areas, FCR is the main source of yield loss. For example, in the northern grains region of Australia (northern New South Wales and Queensland), FCR occurs nearly every year and, on average, affects more than one third of the total cropping area planted, resulting in an estimated average annual yield loss of 4.7% or AUD 41.5 million (Murray and Brennan 2009).

Thus, diseases caused by *Fusarium* species are responsible for significant losses and costs to wheat production on a global scale.

Fusarium Diseases of Wheat

FHB and FCR can occur in most grain growing regions of the world and are caused by a range of *Fusarium* species. Depending on climatic and agronomic factors the dominant *Fusarium* species causing FHB or FCR can vary between geographic regions. Individual strains of the pathogenic species of *Fusarium* differ markedly in pathogenicity, toxigenicity and fungicide sensitivity (Akinsanmi et al. 2004; Nielsen et al. 2011). The incidence of *Fusarium* infection in cereals is increasing worldwide, largely due to the transition away from tillage-intensive production systems, increased use of susceptible cultivars or cereal types, climatic changes, fungicide misuse, and changes in rotational crop preferences (Wu et al. 2014). In wheat, these changes to cropping practices are exacerbating the extent of yield and grain quality losses from FHB and FCR, along with associated increases in mycotoxin contamination due to more frequent and severe FHB epidemics. Several detailed reviews on the disease cycles of FHB (e.g. Goswami and Kistler 2004; Parry et al. 1995) and FCR (e.g. Cook 1980; Alahmad et al. 2018; Kazan and Gardiner 2018) are available and a summary of each disease is presented below.

Fusarium Head Blight (FHB)

At least 17 *Fusarium* species associated with FHB have been identified (Parry et al. 1995). The predominant species are *Fusarium culmorum, F. graminearum*, *F. avenaceum* and *Microdochium nivale,* all of which are also associated with ear, stalk and root rot of maize. These fungi survive in their saprophytic phase within host

residues. Ascospores and/or macroconidia are dispersed from these residues by wind, rain or insects, to infect wheat heads. This usually occurs when warm, wet weather is experienced around anthesis and early grain-filling, so epidemics are often sporadic because disease development depends heavily on environmental conditions which are conducive to disease development during these specific growth stages (Parry et al. 1995). Infected grains are shrivelled and discoloured (white and/or pink), and premature bleaching and death of spikelets or entire heads can occur. Thus, yield loss is primarily caused by floret sterility and poor seed filling. The various causal *Fusarium* species produce different mycotoxins during infection. The mycotoxins can remain stable in stored grain for years (Bockus et al. 2010).

Although the epidemiology and toxigenicity of FHB and FCR are linked, the mycotoxin contamination of wheat kernels is much more significant in the case of FHB (Chakraborty et al. 2006). Trichothecenes associated with FHB are divided into two types; type A trichothecenes, including T-2 and HT-2 toxins, and type B trichothecenes including nivalenol (NIV) and deoxynivalenol (DON) (Miller 2008). Consumption of these toxins can be devastating, for example consumption of contaminated grain in Russia in the 1940s caused the death of nearly 100,000 people (reviewed by Pitt and Miller 2017). Environmental conditions account for approximately half of the variation observed in DON levels in wheat, but variety and cropping history also have a significant impact on final DON levels (Schaafsma and Hooker 2007). Bakker et al. (2018) provided a transdisciplinary overview of mycotoxins produced by *Fusarium* species, including the role of these mycotoxins in pathogen virulence, and several other recent reviews on mycotoxins are available (Miedaner et al. 2017; Pitt and Miller 2017). The provisional maximum tolerable daily intake (PMTDI) of DON in grain (1μg/kg body weight), set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is frequently well exceeded in Africa and parts of Latin America, and has been close to being exceeded in Denmark, Netherlands and Canada (Miller 2008). This highlights the importance of *Fusarium* species not only to food security but also to food safety.

Aside from the health risks associated with mycotoxins, FHB has a twofold negative effect on returns to the producer through reduced yields and discounted price on infected grains. Market discounts in the USA in recent years range from USD 1.84 to 3.67 per tonne per 0.5 ppm of DON in the grain (Dahl and Wilson 2018). The increase in risks and cost to wheat production from mycotoxin contamination are felt throughout the supply chain, from inputs and production on the farm to testing, post-harvest handling, and marketing, whether for export, domestic, food or feed markets.

Fusarium Crown Rot (FCR)

FCR occurs in arid and semi-arid cropping regions around the world including Australia, the Pacific Northwest of the USA, Canada, South Africa, the Middle East, China, North Africa and South America (reviewed by Kazan and Gardiner 2018). Three main species cause FCR, *F. pseudograminearum, F. culmorum* and *F.*

graminearum. Others including *F. avenaceum, F. acuminatum, F. crookwellense* and *F. poae* have also been recorded as causing FCR but are of lesser importance globally due to lower pathogenic virulence or geographical restrictions (Bockus et al. 2010). Typically, hyphae surviving in host residues are the source of inoculum, with infection occurring through the roots, crown, lower culm or leaf sheaths of growing plants when moisture conditions are adequate. Infections result in characteristic browning at the base of infected stems (Wildermuth et al. 1997). The fungus colonises the xylem, causing disruption of water transport within the stem which can lead to premature senescence of heads (ears), resulting in the appearance of white-heads (deadheads) within infected crops (Burgess et al. 2001; Knight et al. 2017). Damage from infection ranges from seedling blight (if infested seed is planted), through post-emergent damping-off, to a reduction in biomass production, grain yield and grain quality (Smiley et al. 2005).

FCR is exacerbated by water stress. Disease development is promoted by warm and dry drought-like conditions post-anthesis (Cook 1980). Once infection has been established, mycelium can colonise the entire stem (Mudge et al. 2006), and can survive in residues for up to 3 years after initial infection (Summerell and Burgess 1988). Thus, the expanding adoption of conservation agriculture (no-tillage with stubble retention) by farmers has largely contributed to an increase in the incidence of FCR globally over the last two decades (Alahmad et al. 2018; Kazan and Gardiner 2018).

FHB and FCR Epidemics: A Global Perspective

Yield Loss Caused by FHB or FCR in Various Cereal-Producing Regions

The following section provides a summary of published reports on FHB (Table 7.2) and FCR (Table 7.3) for different regions and countries, including the causal *Fusarium* species and the extent of estimated yield loss. As highlighted by Savary et al. (2017), quantified data on the impacts of disease on plant health has generally been poorly documented. Thus incidence data has been reported for some regions in the absence of available yield loss data. Sources which determined yield loss to *Fusarium* species in wheat using artificial inoculation have been excluded as they may not be representative of natural field infection.

Table 7.2 The global impact of *Fusarium* head blight (FHB) on wheat production across different growing regions, the causative *Fusarium* species and year(s) when yield loss (%) was recorded

Country	Specific region	*Fusarium* spp.	Year/s	% yield loss	Notes	Source
Oceania						
Australia	Liverpool Plains	*Fg*	1999	NS	2 to 100% incidence	Southwell et al. (2003)
	Southern	*Fg, Fce, Fc, Fp, Fa*	2000	NS	2 to 25% head infection in 39% of fields	Tan et al. (2004)
	Northern	*Fg, Fp*	2010	NS	1.4 to >79% spikelets infected	Obanor et al. (2013)
	All regions	*Fg, Fc*	2008	Est. 0.1%	Nationally	Murray and Brennan (2009)
New Zealand	North Island	*Fg*	1999–2000	NS	% grains infected: 5% *Fg*, 1% *Fa*, 1% *Fpo*	Cromey et al. (2002)
Americas						
Canada	Atlantic provinces	*Fusarium* spp.	1980	30–70%	Spring wheat	Martin and Johnston (1982)
South America	Paraguay	*Fg*	1972–75	70%	(up to)	In: Bekele (2018)
	Uruguay	*Fg*	1977	Est. 44%	Nationally	Diaz de Ackermann and Kohli (1997)
			1991–93	0.5–31%		
	Argentina	*Fg*	1993	50%	Durum wheat	de Galich (1997)
			NS	Est. 20–30%	Historical ave.	

(continued)

Table 7.2 (continued)

Country	Specific region	*Fusarium* spp.	Year/s	% yield loss	Notes	Source
USA	Indiana	*Fg*	1928	15%	Early record	Mains and Curtis (1929)
	All regions	*Fg*	1982	4%	Nationally	In: Parry et al. (1995)
	SWR growing areas	*Fusarium* spp.	NS	Est. 25%	For 15 million acres SRW wheat	Kephart (1991)
	North Dakota	*Fusarium* spp.	1993	45%	70% FHB-affected kernels	In: McMullen et al. (1997)
	Minnesota	*Fusarium* spp.	1993	40%	North-west region (70% production area)	In: Windels (2000)
	North Dakota	*Fusarium* spp.	1998–2000	NS	1.3 Mt	Nganje et al. (2004)
	Virginia	*Fusarium* spp.	2003	54%	% loss compared to 10-year average	Cowger and Sutton (2005)
	North Carolina	*Fusarium* spp.	2003	52%	% loss compared to 10-year average	Cowger and Sutton (2005)
	Maryland	*Fusarium* spp.	2003	28%	% loss compared to 10-year average	Cowger and Sutton (2005)
	Nebraska	*Fusarium* spp.	2008	2.3%	State-wide total (up to 20%)	Lilliboe (2008)
	Kansas	*Fusarium* spp.	2008	1.9%	State-wide total (Eastern areas: 8.75–17.6%)	Lilliboe (2008)
	Mid-South & South eastern states	*Fusarium* spp.	2009	50%	Anecdotal reports	In: McMullen et al. (2012)
Europe						
Europe	All regions	*Fusarium* spp.	<1998	Ave. 10–30%		In: Bottalico (1998)
Russia	Krasnodar	*Fusarium* spp.	NS	25–50%	(up to)	In: Bottalico (1998)
Hungary	NS	*Fusarium* spp.	1970	Est. 40–50%	In some regions	In: Parry et al. (1995)
Romania	NS	*Fg, Fc, Fa*	1976–77	Est. 40%	In some regions	Tușa et al. (1981)
Italy	Bologna	*Fg, Fc, Fpo*	1995–2001	NS	Isolate frequency (durum wheat): 32.1% *Fg*, 25.2% *Fc*, 17.8% *Fpo*, 24.9% other	Pancaldi et al. (2010)

(continued)

Table 7.2 (continued)

Country	Specific region	*Fusarium* spp.	Year/s	% yield loss	Notes	Source
Sweden	South, West & East	*Fpo, Fa, Fg, Fc*	2009–11	NS	Isolate frequency (grain): 100% *Fpo*, 99% *Fa*, 90% *Fc*, 75–90% *Fg*	Lindblad et al. (2013)
Asia						
China	NS	*Fusarium* spp.	1950–90	Ave. 10–20%	Up to 40% in severe epidemics	In: Wang (1997)
	North China	*Fg, Fp*	NS	NS	12–13 Mt	Ji et al. (2016)
	Huai & Yellow rivers	*Fg*	1998	100%	In some fields (average 80% scabbed spikes)	In: Yao et al. (2008)
	Yangtze Valley	*Fusarium* spp.	1965–95	30–40%	(up to)	In: Parry et al. (1995)
		Fg	2003	>20%	60–80% scabbed spikes	In: Yao et al. (2008)
	Sichuan	*Fg*	2004	NS	292,000 ha affected (10–61% scabbed spikes)	
	Hubei Province	*Fg*	2006	NS	266,700 ha affected	
	Henan Province	*Fg*	2006	NS	885 million kg	
India	Arunachal Pradesh	*Fa*	1980–82	15–29%	Random field survey, 50% infected heads	Chaudhary and Edison (1991)
	Punjab	*Fusarium* spp.	2004–05	NS	Incidences up to 90%	Teli et al. (2016)
Africa						
Kenya	Nakuru district	*Fusarium* spp.	2006	NS	Ave. incidence 7% and severity 24%	Muthomi et al. (2007)
South Africa	NS	*Fusarium* spp.	2006	NS	8–27% heads infected, up to 74% incidence	Scott and de Jager 1988
	Central region	*Fg, Fc, F. crook-wellense*	2007	22–24% 7–17%	Highly susceptible cultivars Less susceptible cultivars	Kriel and Pretorius (2008)
Tunisia	Northern	*Fc*	2004	NS	Disease incidence 17–56.9%	Fakhfakh et al. (2011)
			2007	NS	Disease incidence 33.2–86.3%	

Abbreviations: average (av.), estimated (est.), not specified (NS), *Fusarium avenaceum (Fa), F. culmorum (Fc), F. cerealis* (*Fce*), *F. graminearum (Fg), F. poae* (*Fpo*), *F. pseudograminearum (Fp),* Megatonnes (Mt), soft red winter (SRW)

Table 7.3 The global impact of *Fusarium* crown rot (FCR) on wheat production across different growing regions, the causative *Fusarium* spp. and year(s) when yield loss (%) was recorded

Country	Region	*Fusarium* spp.	Year/s	% yield loss	Notes	Source
Oceania						
Australia	Southern Qld	*Fp*	<1980	Ave. 5%	Up to 26%	Burgess et al. (1981)
	Northern NSW	*Fp*	1978–81	Ave. 19%	Range 0–89%	Klein et al. (1991)
	Victoria	*Fp, Fc*	1997–2008	Ave. 2–3%	Total annual loss (>5% in 10–20% fields)	Hollaway and Excell (2012)
	All regions	*Fp, Fc, Fg*	2008	Est. 4.8%	Total loss across Australia	Murray and Brennan (2009
	South Australia & Victoria	*Fc, Fp*	2005–10	8–36% 24–52%	Bread wheat Durum wheat	Hollaway et al. (2013)
	Merredin, WA	*Fp*	2014	NS	30–50% of fields impacted	Hüberli et al. (2017)
New Zealand	South Island	*Fc, Fp*	2003	NS	Incidence: 16% *Fc*, 1.5% *Fp*	Bentley et al. (2006)
Americas						
Chile	Southern Chile	*Fa, Fg, Fc*	2011–12	NS	Ave. 13.9% incidence, 11.3–80% severity	Moya-Elizondo et al. (2015)
USA	Pacific Northwest	*Fc*	1960s	17–50%	Compared to uninfected fields	Cook (1968)
		Fp, Fc	1994	Ave. 9.4%	Up to 35%	Smiley et al. (2005)
		Fusarium spp.	1994	18% 3%	For "heavily" infected fields For "lightly" infected fields	Paulitz et al. (2002)
		Fp, Fc	2008–09	NS	98% incidence	Poole et al. (2012)
	Montana	*Fusarium* spp.	2008–09	25–35%	51% incidence	Moya-Elizondo et al. (2011)
Asia						
China	North China Plain	*Fp*	2013–16	NS	Incidence: 14.9% in roots, 27.8% in stems	Xu et al. (2018)
	Hebei Province	*Fp, Fg*	2017	NS	75–100% incidence	Ji et al. (2018)

(continued)

Table 7.3 (continued)

Country	Region	*Fusarium* spp.	Year/s	% yield loss	Notes	Source
Iran	North West	*Fp*	1999–2004	18–46%	Field survey of four provinces	Saremi et al. (2007)
	42 regions	*Fc, Fp*	2010–11	NS	Isolate frequency: 51% *Fc*, 18% *Fp*, 9% *F. equiseti*, 22% other	Pouzeshimiab et al. (2012)
Nepal	Bhairahawa	*Fusarium* spp. + *Bs*	1991	Est. 4%	FCR + CRR	Dubin and Bimb (1994)
Pakistan	Punjab Province	*Fusarium* spp.	1999	NS	37% incidence	Iram et al. (2003)
Africa						
Tunisia	NS	*Fusarium* spp.	1974	44%		Ghodbane et al. (1974)
		Fc	NS	NS	Incidence: 2–32% plants	Gargouri et al. (2012)
Morocco	Central West	*Fc* + *Bs*	1992–93	NS	Up to 30% white heads (natural infection)	Mergoum et al. (1997)

Abbreviations: average (av.) *Bipolaris sorokiniana (Bs)*, common root rot (CRR), estimated (est.), *Fusarium avenaceum (Fa), F. culmorum (Fc), F. graminearum (Fg), F. pseudograminearum (Fp)*, *Fusarium* crown rot (FCR), not specified (NS)

Americas

F. graminearum is the dominant species causing FHB in North America, decreasing yield and grain quality in hard red spring, soft red winter, durum wheat, and barley. Weather conditions in upper Great Plains states, such as North Dakota and Minnesota, are often favourable for FHB development, and severe epidemics have been reported in these states multiple times in the past two decades. In 2003, FHB caused estimated losses of 54.2% in Virginia, 52.0% in North Carolina and 28.3% in Maryland (Table 7.2). In 2008, parts of Nebraska experienced 20% yield loss to FHB, and in 2009 anecdotal reports of 50% yield loss were observed in several mid-south and south-eastern states (reviewed by McMullen et al. 2012). These losses have significant economic consequences. For example, between 1998 and 2000 it is estimated that FHB caused the loss of 1.3 Mt of wheat which led to direct economic losses of USD 734.3 million (the majority of this loss occurred in North Dakota) and secondary impacts of approximately USD 1809.3 million (Nganje et al. 2004).

In Canada, FHB has been responsible for severe epidemics since the 1980s with 30–70% yield loss reported in spring wheat (Table 7.2). In response to increasing FHB epidemics and the risk of grain mycotoxin contamination, the Canada Grains Commission has publicly recorded FHB incidence and severity since 2003 for hundreds of fields of Canada Western red spring wheat for each of the major wheat-producing regions (see https://www.grainscanada.gc.ca/str-rst/fusarium/data/

frequency-en.htm). Pathogenicity testing has revealed that *F. culmorum* and *F. graminearum* are the most prevalent pathogenic *Fusarium* species associated with cereals in Canada (Fernandez and Chen 2005).

In South America, all wheat grain samples tested by Tralamazza et al. (2016) collected from important wheat-growing regions in Brazil were contaminated by DON-producing *F. graminearum*. This is a concern, given the increasing production of wheat occurring in Brazil in recent decades (USDA 2014). In Uruguay, FHB is one of the most destructive diseases of wheat; serious epidemics since the late 1970s have caused up to 44% yield loss (Diaz de Ackermann and Kohli 1997) (Table 7.2). Grain testing showed that 48% of wheat grain samples from Uruguay had detectable levels of DON in 1993–95 (Pineiro 1997). Yield loss to FHB in Argentina during epidemics has historically been around 20–30%, and as high as 50% in 1993. These outbreaks significantly decreased plantings of durum wheat (from 20% down to 3.5%) in subsequent seasons; durum was replaced by more resistant bread wheat varieties (reviewed by de Galich 1997).

The population structure of *F. graminearum* in the Americas has been reported to have changed over the past two decades (Ward et al. 2008), possibly influenced by increased maize plantings, climate change and reduced tillage practices (Nielsen et al. 2011). A new type A trichothecene, named NX-2, was recently discovered and has been detected only in the USA (Varga et al. 2015). It remains to be determined how frequently NX-2 is occurring naturally in contaminated grain, and whether screening methods should be adapted to include detection of this new toxin. The *Fusarium* species causing FHB and mycotoxins associated with them are variable; the dominant mycotoxins vary from region to region (e.g. Ward et al. 2008, Schmale et al. 2011, Tralamazza et al. 2016). Monitoring shifts in *Fusarium* populations and the mycotoxins they produce is important from the perspective of global food security and safety.

The higher incidences of FHB have influenced planting decisions, particularly in the last 20 years. In North America, the area planted to wheat is at its lowest level in 50 years, down from 70 million acres in mid-1990s to 49 million acres in 2016, which has been associated with several severe FHB epidemics and the emergence of DON issues in harvested grain (Dahl and Wilson 2018). In response, soybean and maize production has increased in the USA. In Canada, growers are also planting less wheat as a result of increasing risk from FHB, and have adopted canola as an alternative, lower-risk crop (Bianchini et al. 2015).

Although FCR is not considered as large a problem to wheat production in the USA as FHB, there is still potential for the disease to cause substantial yield loss under favourable conditions, for example in the Pacific North West (Table 7.3). South America is also affected by FCR. In a field survey in Chile in 2011, 13.9% of wheat stems were infected with *F. avenaceum, F. graminearum* or *F. culmorum*, with FCR severities between 11.3 and 80% in the 48 commercial fields surveyed (Moya-Elizondo et al. 2015). In Canada, *F. pseudograminearum* causing FCR had an incidence of 12% in a naturally infected field trial over the years 2000–2003 (Fernandez and Zentner 2005).

The USA is the world's second largest exporter of wheat grain after Russia, exporting 24 Mt of wheat in 2016 (Table 7.1). In developing regions (including South America) which rely on this imported grain for food, any unexpected increase in grain prices associated with declining production due to increased risk from disease can be detrimental to food security, intensifying poverty, hunger and political unrest.

Europe

Fusarium species are widespread pathogens of cereals in Europe and have been estimated to cause 10–30% reduction in yield (reviewed by Bottalico 1998). Wheat yields per unit area in Europe are well above the worldwide average (Oerke et al. 2012), due to favourable climatic conditions such as increased levels of in-crop rainfall and lower temperatures during grain-filling. Consequently, FHB is of much more concern than FCR to wheat production throughout Europe, although FCR occurs frequently in some Mediterranean regions e.g. Sardinia (Balmas et al. 2015).

F. culmorum has been the dominant species causing FHB in cooler areas (e.g. France, Poland, Finland, and the Netherlands), but warmer summers are shifting the species dominance towards *F. graminearum* (Miller 2008; Nielsen et al. 2011; Parikka et al. 2012). *F. graminearum* is the dominant species in other areas such as Switzerland, Italy and France, but *F. poae* and *F. avenaceum* are also important (e.g. Sweden, Table 7.2) (Ioos et al. 2004; Karlsson et al. 2017; Lindblad et al. 2013; Pancaldi et al. 2010; Schoneberg et al. 2018). These pathogens cause FHB every year in some regions (e.g. Bologna, Italy) (Pancaldi et al. 2010) and DON is commonly detected in wheat grain from Europe (Bottalico 1998; Lindblad et al. 2013). Trichothecene genotypes in Europe are primarily 15-ADON (82.9%), 3-ADON (13.6%) and NIV (3.5%) in *F. graminearum* populations, and 3-ADON (59.9%) in *F. culmorum*, with NIV accounting for the remaining 40.1% (Pasquali et al. 2016). Higher DON concentrations have been associated with maize-wheat rotations (Bottalico 1998).

Russia and Bulgaria experience widespread loss to FHB with high levels of DON contamination in wheat grain (Bottalico 1998; Vrabcheva et al. 1996). Severe epidemics, caused mainly by *F. graminearum,* have occurred in the south of Russia since the late 1980s (Ablova and Slusarenko 1997). For example, FHB has been reported to cause 25–50% yield loss in wheat grown in Krasnodar, Russia (Table 7.2). Losses of this magnitude have major implications for trade, as Russia is the largest exporter of wheat with over 25 Mt of wheat exported in 2016 (Table 7.1).

In Denmark, FHB is generally not considered a serious threat to wheat production because the farming system (only 3% wheat-wheat and < 1% wheat-maize rotations (Nielsen et al. 2011)) is not conducive to significant disease development and mycotoxin levels are consistently below permitted limits for DON. However, *Fusarium* species are still commonly recovered from wheat grain and FHB can cause isolated issues in some seasons (Nielsen et al. 2011).

Europe is one of the largest exporters of wheat with Russia, France, Germany and Ukraine together responsible for more than 35% of all world wheat exports in 2016 (Table 7.1). Like North America, the impact of *Fusarium* species on cereal production and food security in Europe has more of a supply-chain effect to countries in South America, Africa and Asia that rely on imported wheat.

Asia

Wheat production in China has increased roughly five-fold since the 1960s and now almost equals rice, being grown on some 30 million hectares (Miller 2008). FHB affects around 25% of this wheat-growing area in more than 20 provinces (Wang 1997; Yao et al. 2008). *F. graminearum* is the most common pathogen causing FHB in North China where wheat-maize rotations dominate, but *F. pseudograminearum* has also recently been reported to cause FHB in this area (Ji et al. 2016; Xu et al. 2015). The rice-associated *F. asiaticum* is the dominant species causing FHB in Southern China where wheat-rice rotation is more common (reviewed by van der Lee et al. 2015). FHB epidemics are most frequent along the Yangtze River, for example in the Jiangsu and Zhejiang Provinces (Gale et al. 2002). In China alone, more than 1 Mt of wheat can be lost during an FHB epidemic, with yield loss in severe epidemics around 10–40% but up to 100% in some fields (Table 7.2). Large scale toxicoses (>50,000 people) have been reported in China and India, highlighting the further threat of FHB to food safety in Asia (Zhuping 1994).

FHB has historically not been of great importance in India, but epidemics are increasing in frequency, perhaps due to changing climatic conditions. Field surveys from the 1980s reported up to 50% incidence of head infections and FHB-affected grains in Arunachal Pradesh in north-east India (Chaudhary and Edison 1991). More recently, incidences of up to 90% were recorded in Punjab in durum wheat in the particularly wet 2004–2005 season, causing devastating economic and yield losses (reviewed by Teli et al. 2016).

In Turkey some areas have reported up to 43% yield loss from FCR in bread wheat (Hekimhan et al. 2004). Given that Turkey produces approximately 20 Mt of wheat per year these losses have major impacts on local food security (Nicol et al. 2012). Of additional concern is that Turkey produces 4–6 Mt of durum wheat which is highly susceptible to FCR damage (Nicol et al. 2012). In a nationwide survey of wheat crown and root rots more than 26% of fields had one or more fungal species causing FCR, the most prevalent being *F. culmorum* (Tunali et al. 2008).

The first report of *F. pseudograminearum* causing FCR in China was in 2011 in Henan, China's largest wheat-growing area (Li et al. 2012). FCR is currently seen as an "emerging" disease in China, with incidence and severity increasing steadily in response to increased wheat plantings (Ji et al. 2018). A survey of 191 fields in Hebei province in 2017 showed that 75–100% of sites experienced FCR, caused predominantly by *F. pseudograminearum* (southern-dominant) and *F. graminearum* (northern-dominant) (Ji et al. 2018). Wheat-growing regions like Henan experience

conditions conducive to FCR development, suggesting that FCR will be increasingly important in Chinese wheat production (Li et al. 2012).

Of all the continents, Asia has the largest number of undernourished people. The food crisis in Yemen is the largest in the world, affecting more than 10 million people (FSIN 2018). Wheat is the most important crop in western Asian countries such as Iran and Iraq, and production is constrained by FCR (Safaee et al. 2012). However, primary research into root and crown diseases has only recently commenced in these areas (Hameed et al. 2012; Safaee et al. 2012). Accelerating research and development of management strategies to limit the impacts of FCR, and in some instances FHB, therefore appears to be a priority in Asia, as a contribution to global food security.

Africa

F. graminearum is the most common and most pathogenic species causing FHB in Africa (Minnaar-Ontong et al. 2017; Muthomi et al. 2007). FHB is of increasing concern due to the expanding production of irrigated spring wheat in South Africa (Sydenham and de Villiers 2014), increased adoption of maize-wheat or medic-wheat ions, lack of available resistant cultivars and no locally-registered fungicides for the control of FHB (de Villiers 2014; Klaasen et al. 1992). Early field surveys of FHB in South Africa report on average 8% to 27% head infection with disease incidences as high as 74% (Scott and de Jager 1988). In Kenya, a survey in 2006 revealed that FHB occurred in 90–100% of wheat fields, with an average severity of 24% (Table 7.3) (Muthomi et al. 2007). In response to the increasing threat of FHB, many wheat growers in Africa have reverted back to burning and ploughing of residues (Kriel and Pretorius 2008).

FCR in Morocco is caused by *F. culmorum* and is a major constraint to dryland wheat production which has increased due to the high plantings of durum wheat (around 25% of the total cereal growing area) (Mergoum et al. 1997) and increased adoption of wheat-maize rotations (Brahim 2012). *F. culmorum* is also the main causal agent of FCR in Tunisia, causing significant yield loss in durum wheat which is the most common type of wheat grown in this area (>40% of cereal cropping area) (Fakhfakh et al. 2011). A high proportion of wheat stubble is grazed by sheep in Tunisia which reduces straw residues, but the incidence of *F. culmorum* infection in fields remains as high as 32% which indicates that inoculum survives below the soil surface to cause subsequent FHB (Table 7.2) and FCR (Table 7.3) infections (Fakhfakh et al. 2011; Gargouri et al. 2012).

Africa has the highest prevalence of undernourished people in the world, with 32 million people facing acute food insecurity (FSIN 2018). In 2003–04 the unfavourable season resulted in a cereal deficit of 4 Mt across Africa with emergency food aid required (Stewart 2003). Given that *Fusarium* species are widespread and can cause considerable yield loss in Africa (Tables 7.2 and 7.3), FHB and FCR epidemics could easily be responsible for future food deficits and increased food insecurity

in Africa. There are also food safety concerns around FHB, for example in South Africa mycotoxin levels are well above PMTDI (Miller 2008). Further to this, it is not known whether there are interactions with malnutrition and mycotoxin exposure which could be causing greater health implications than previously thought (Wu et al. 2014).

Oceania

F. pseudograminearum is the main causal species of FCR across the Australian wheat-belt (Akinsanmi et al. 2004; Backhouse et al. 2004; Khangura et al. 2013) with *F. culmorum* also being an important species in the higher rainfall areas of eastern Australia (Backhouse et al. 2004). Losses of up to 89% from FCR have been recorded in individual wheat fields (Klein et al. 1991). Comparisons of yield losses to FCR in Australia were conducted by Murray and Brennan (2009) between 1988, 1998 and 2008 and show an increase in yield loss in all grain growing regions. In the northern region (northern NSW and Queensland) the potential yield loss to FCR has increased dramatically from 3% in 1988, 13% in 1998 up to 22.2% in 2008. The use of durum wheat has subsequently declined in recent seasons, because of its greater susceptibility to FCR than bread wheat and barley. Australia has a demand for high-protein wheat grain for export markets, which requires greater input of nitrogen fertilisers (mainly urea); this has recently been shown to increase the risk of infection and yield loss from FCR (Simpfendorfer, unpublished data). With FCR, inoculum build-up and disease development is favoured in no-till systems where inoculum becomes concentrated in the previous year's cereal rows (Verrell et al. 2017). This has caused an increase in FCR levels in all Australian cropping regions, as growers have progressively adopted no-till stubble retention cropping systems (Alahmad et al. 2018). The recent increase in yield loss to FCR across Australia is concerning and could be a reflection of climate change, given that FCR is favoured by drier soils, warmer conditions and intermittent rainfall events, especially during anthesis and grain-filling (Wallwork 2000).

FHB affects wheat production in New Zealand, with the highest incidence of *Fusarium* infected grain coming from fields growing maize in the previous season (Cromey et al. 2002). *F. graminearum* and *F. culmorum* are the predominant species in the North Island, while *F. avenaceum* predominates in the South Island (Cromey et al. 2002), with grain infection levels being lower overall on the South Island (Sayer and Lauren 1991). NIV and DON chemotypes are equally common in New Zealand (Lauren et al. 1992). FHB in Australia has been reported sporadically, but extensive wet weather in 2010 and 2016 caused epidemics in the Northern Region primarily associated with basal FCR infections by *F. pseudograminearum* (Obanor et al. 2013; Simpfendorfer et al. 2017).

In Australia, yield loss from FCR in wheat has a localised economic effect through reduced grain availability for export markets. Over the last decade, Australia has only required approximately 2 Mt of wheat for domestic food consumption

while between 14 and 25 Mt is exported annually (FAO 2018). Improving grain yields in countries like Australia which have large areas of land available and where grain yields are relatively low could be an effective strategy for increasing global wheat production (Curtis and Halford 2014). Thus, with *Fusarium* species capable of causing up to 25% potential yield loss in years conducive to FCR (Murray and Brennan 2009), continued focus on lowering these losses in Australia would be beneficial for global wheat production.

Impact on Food Security

Regions relying on a significant proportion of dietary energy from cereals (>50%), including wheat, and with a high prevalence of undernourishment (e.g. Africa and Asia) are at high risk of food security impacts from losses to FHB and FCR (Table 7.4). Regions with lower dietary energy supply from cereals and lower levels of undernourishment (e.g. Oceania, North America and Europe) have a reduced risk of food security impacts from losses to FHB and FCR. However, severe economic impacts can be experienced following epidemics in these areas (Nganje et al. 2004). Furthermore, yield losses from FHB and FCR in these regions are likely to affect grain export supply and global pricing negatively, as these regions are major global exporters of wheat (Table 7.1). Consequently, losses from FHB and FCR in regions such as Oceania, North America and Europe are likely to have implications for food security in regions that rely heavily on cereal imports such as South America (Latin America and the Caribbean) and Africa (Table 7.4).

The impact of any disease on food security is difficult to assess, as it depends on complex interacting epidemiological (e.g. disease interactions), agricultural and economic aspects (Savary et al. 2017). Up-to-date and quantitative data for yield

Table 7.4 Total dietary energy supply derived from cereals (%), dependency on imported cereal for food (cereal import dependency ratio %), and ability to access food (% of total population considered undernourished) for major regions of the world

Region	Dietary energy supply derived from cereals (%)	Cereal import dependency ratio (%)	Prevalence of undernourishment (%)
Africa	55	25	18.6
North America	24	−23.8	<2.5
South America	39	32.5	6.4
Europe	34	−8.2	<2.5
Asia	55	8	12.9
Oceania	21	−184.7	5.5
Total	50	N/A	11.3

Data are estimates from FAO averaged over three years (2011–2013) (retrieved on 09/19/2018 from http://www.fao.org/faostat/en/#data/FS)

loss resulting from FHB and FCR is lacking for many regions (Table 7.2 and 7.3). In many cases, the method of yield loss estimation is vague (e.g. whether confounded by artificial inoculation), or it applies only to a specific localised area, or was estimated some time ago and may not reflect actual values under current cropping practices and changing climatic conditions. Furthermore, field trial data may over- or under-estimate crop losses at a field scale. There is a need for more data and other information (e.g. from unpublished trials) to be made available, perhaps by coordinated effort like that for the chemotype dataset for Europe (Pasquali et al. 2016). Then the impact of FHB and FCR on wheat could be more thoroughly assessed on a global scale, leading to improved estimates of the implications for current and future food security.

Reducing the Impact of FHB and FCR in Wheat: Management and Limitations

Due to the relatedness of the pathogens causing FHB and FCR, some management strategies are effective at reducing inoculum and/or yield losses to both diseases. As *Fusarium* species can infect a wide range of cereals, rotation with non-host crops is an effective strategy for reducing pathogen levels in wheat (Dill-Macky and Jones 2000). Tillage accelerates decomposition of crop residues, reducing the survival of species of *Fusarium* (Bockus et al. 2010; Dill-Macky and Jones 2000; Hollaway et al. 2013; Pereyra et al. 2004; Verrell et al. 2017). Reducing the amount of residue retained on the soil surface can limit disease incidence (Smiley et al. 1996). Disturbing infected residues through tillage close to sowing, with insufficient time for decomposition, can result in increased incidence of FCR and associated yield penalties (Verrell et al. 2017). Controlling grass weed hosts and burning of infected residues can also reduce disease development, but the practice of burning has been much reduced in the USA and Australia by awareness of its negative environmental impact (Burgess et al. 2001).

Specific risk-reducing technologies for FHB include the application of fungicides at the start of anthesis and growing moderately resistant varieties. These practices used together have an additive effect and can decrease incidence and the risk of DON contamination in the wheat supply chain by as much as 50% (Bianchini et al. 2015; Dahl and Wilson 2018). In response to increasing concerns over FHB, fungicide use in the USA has increased from almost zero in the 2000s to as high as 75% of the area planted to hard red spring wheat (Dahl and Wilson 2018). The application of the fungicide combination tebuconazole + prothioconazole has been extensively shown to reduce the severity of FHB in wheat globally (Haidukowski et al. 2012; Paul et al. 2008; Simpfendorfer 2016b; Yoshida et al. 2012). Although DON levels are most effectively reduced by fungicide application at anthesis (Pirgozliev et al. 2008), application at late-milk stage (20 days post-anthesis) may still reduce mycotoxin accumulation in grain (Yoshida et al. 2012).

FHB resistance types I and II have been identified, with numerous quantitative trait loci (QTLs) incorporated into breeding lines (Li et al. 2015). Single QTLs do

not confer total protection against FHB in wheat, and it may become difficult to integrate other desirable agronomic and quality traits (Miedaner et al. 2017). Despite the relatedness of the causal species of *Fusarium*, FHB resistance does not necessarily confer resistance to FCR (Li et al. 2010). Partial FCR resistance, characterised by reduced disease symptoms or reduced fungal biomass, has been identified in wheat cultivars in Australia and the USA (reviewed by Kazan and Gardiner 2018). Ma et al. (2010) reported a QTL, *Qcrs.cpi-3B*, which reduced FCR disease severity caused by either *F. graminearum* or *F. pseudograminearum* by up to 42% and it appears to be a worthy target for future breeding efforts. It may also be possible to use gene-editing techniques such as CRISPR to enhance resistance (Duba et al. 2018) .

Although resistant cultivars are not yet available for FCR (Alahmad et al. 2018), cultivar choice is still important for reducing yield loss to FCR and avoiding inoculum build-up. In Australia over the last decade there has been an increased breeding focus on the relative yield performance of wheat cultivars in the presence of FCR infection. At high infection levels, the yield benefit of growing the best cultivars increased from 5–10% in 2007 to around 20–30% in 2013–14 (Simpfendorfer 2016a). In a no-till system, sowing into clean inter-row spaces can increase wheat yield by 6% (0.14 t/ha) compared to sowing onto a previous wheat row, through avoiding contact with existing *F. pseudograminearum* inoculum (Verrell et al. 2017).

The control of FCR using in-crop fungicide application is of limited value compared to technologies available for FHB management (Burgess et al. 1975; Kazan and Gardiner 2018). Fungicides can be used to reduce *Fusarium* seedling blight to some extent, but yield benefits beyond the seedling stage have not been evident in Australia (reviewed by Alahmad et al. 2018). One product, Rancona® Dimension (ipconazole + metalaxyl by Arysta LifeScience), is registered for the control of FCR in Australia but has shown limited efficacy in reducing yield loss when used in isolation (Simpfendorfer 2016c). A number of biocontrol agents, including various antagonistic bacteria (Huang and Wong 1998), *Trichoderma*, and *Fusarium* species (Lakhesar et al. 2010), have been identified which displace or supress the growth of *Fusarium* species under laboratory or glasshouse conditions, but field validation of yield benefits is largely lacking.

Due to the complex nature of FHB and FCR and the limitations for their management, interest in novel control methods is growing. For example, microwaves are being explored as a rapid heat-kill technique to reduce *F. pseudograminearum* inoculum in wheat residues, although this technology has yet to be validated in the field (Petronaitis et al. 2018). Transgenic wheat expressing a glucosyltransferase from barley (*Hordeum vulgare*), *HvUGT13248,* which confers enhanced plant resistance to FHB when infected with *F. graminearum*, appears promising (Li et al. 2015). Many toxins and secondary metabolites produced by *Fusarium* species have been discovered in recent times (Kazan and Gardiner 2018) and analysis of these may provide insights into pathogen virulence or novel avenues for control. A new sequence-based community profiling methodology for FHB can analyse the diversity of *Fusarium* species associated with different hosts, chemotypes and agricultural practices alongside other crop-associated fungi (Walder et al. 2017). This will be especially useful in diagnostics, in forecasting epidemics, and in temporal and

spatial monitoring of toxigenic *Fusarium* species. Biotransformation of *Fusarium* toxins may also offer a novel approach to improving plant resistance (Bakker et al. 2018).

Prediction models are an important tool which has allowed decision-makers to manage FHB more effectively in wheat, for example in timing of fungicide applications or in making harvest decisions. Models for predicting levels of DON in grain take account of rainfall timing, temperature and water stress (Miller 2008); for example the DONcast model has been used commercially on wheat in Canada for over ten years (Schaafsma and Hooker 2007). Models to predict FHB epidemics are available (Carranza et al. 2007; De Wolf et al. 2003; Del Ponte et al. 2005). However, modelling has limitations and may be restricted to areas with online meteorological data (Schaafsma and Hooker 2007).

A quantitative PCR-based soil testing service called PREDICTA® B, offered by the South Australian Research Development Institute (SARDI) uses inoculum concentrations in soil and cereal residues to estimate the risk of soil-borne disease development and to predict yield loss from FCR (Hollaway et al. 2013). This has particular value when infected but symptomless plants make unrecognised contributions to disease reservoirs, with the potential to damage successive wheat crops (Klein et al. 1991). PREDICTA® B has particular value in regions where FHB and FCR are endemic, as symptoms are not always detected even when infection is sufficient to cause yield loss (Smiley et al. 1996). Similarly, mycotoxin contamination can occur in asymptomatic crops, so mycotoxin testing may be important even when FHB symptoms are not evident (Imathiu et al. 2013; Schoneberg et al. 2018).

Assays have been developed to characterise *Fusarium* chemotypes (Schmale et al. 2011). They need further development as new toxins are discovered, such as NX-2 in *F. graminearum* (Varga et al. 2015). The distribution of *Fusarium* species and strains has been well documented in some regions; however chemotype assessments remain to be conducted in most regions. The chemotype map produced for Europe by Pasquali et al. (2016) could be used as a model for collaborative studies across other regions.

Reducing yield loss to *Fusarium* species will be an ongoing challenge as climate and cropping systems change (Bakker et al. 2018), particularly since attainable wheat yields will shift with climate change (Godfray et al. 2010).

Recent Changes to Farming Systems Affecting FHB and FCR Epidemiology

Climate Change

Global wheat production has been identified as being vulnerable to climate change, and in areas such as southern Asia wheat yields are predicted to decline by 20–30% by 2050 (USAID, 2018). Rising temperatures and changes to weather patterns and water availability are already having a negative effect on plant development and

yield (Bita and Gerats 2013). The potential effect of high temperatures on crop yield has been reviewed previously and is likely to pose a significant threat to global food security (Bita and Gerats 2013; Christensen and Christensen 2007; Curtis and Halford 2014; Vermeulen et al. 2012). Reductions are predicted in grain yield and in grain quality attributes such as reduced protein levels; food safety issues are also anticipated, such as elevated levels of mycotoxin contamination due to FHB (Chakraborty and Newton 2011; Vermeulen et al. 2012).

The frequency of extreme fluctuations in climatic conditions such as very wet or dry growing seasons is increasing worldwide (Zhang et al. 2007). Given sufficient inoculum, extreme conditions during the wheat growing season may favour either FHB (wet, humid conditions) or FCR (dry conditions). Droughts combining rain deficit and high temperatures in Australia in 2006 and Russia in 2010 were associated with significantly reduced wheat yield across large areas, with a large impact on export supply and grain prices globally (Curtis and Halford 2014).

Under FCR pressure, increased heat and moisture stress during grain filling can result in up to 55% yield loss in wheat in semi-arid regions such as Australia; only 3% yield loss occurred under the same infection levels when lower temperatures were experienced during grain-filling and moisture stress was avoided by irrigation (Simpfendorfer and Gardner 2013). Hence, losses to FCR globally are likely to be exacerbated in regions predicted to have increased temperatures during grain-filling and greater frequency of water stress: for example in Turkey, Africa, Australia, China, Iran and the Pacific north-west of the USA (Alahmad et al. 2018; Zhang et al. 2007).

By contrast, predicted higher temperatures and humidity and increased rainfall are likely to favour FHB development, and thus the spread of FHB in regions such as Europe, Canada, India and parts of the USA (Bianchini et al. 2015; Chakraborty and Newton 2011; Miller 2008). Changes to wheat phenology due to climate change could synchronise *F. graminearum* inoculum production with flowering of the crop (Miller 2008; Vaughan et al. 2016). Inoculum levels are predicted to increase under elevated CO_2 conditions, and reduce the benefit of partially resistant varieties (Melloy et al. 2010). A shift in species composition is also likely, for example an increase in the prevalence of *F. langsethiae* and *F. poae*, which prefer dry conditions, in Europe (Parikka et al. 2012).

Increasing maximum temperatures are also predicted to reduce crop water-use efficiency (Shah and Paulsen 2003). Water scarcity has been identified as a major threat to grain production and food security (Hanjra and Qureshi 2010). Cereal production could be severely restricted in arid and semi-arid wheat-growing regions, for example in China, parts of the USA and Australia (Lang 2010). Water stress could present a risk of greater impact from FCR. High priority is attached to the development of drought-tolerance in wheat, and this may lead to increased tolerance of FCR (Alahmad et al. 2018). In glasshouse experiments with *F. pseudograminearum* there is evidence that increasing temperatures may reduce pathogen fitness (Sabburg et al. 2015), but these findings require field validation.

Cropping Practices

Wheat yield per hectare is estimated to have approximately doubled since the early 1960s, mainly due to increased use of fertilisers, more irrigation, and availability of more productive cultivars. In response to these increasing wheat yields, use of pesticides (including fungicides) has increased 15–20-fold, yet yield losses to FHB have increased during this period (Oerke 2006). Thus, management of the vulnerabilities associated with increasing wheat yield is important if ambitious future wheat production targets are to be met.

Changes to cropping practices such as reduced crop rotation, reduced tillage and increased retention of cereal stubble can increase inoculum levels of various soil- and stubble-borne pathogens including the *Fusarium* species that cause FHB and FCR (Yudelman et al. 1998). Kassam et al. (2015) estimated that the area of conservation agriculture practised globally in 2013 was around 157 million ha, about 11% of the global cropping area. This represents an increase of 47% globally since 2008–09 when the estimate was 106 million ha. In the USA alone, the area under conservation agriculture has increased by approximately 25%. This, in combination with the use of susceptible cultivars (e.g. soft white winter wheat) into new areas, and the increased acreage of maize is likely to be associated with the observed increase in DON levels in wheat grain in the USA (Bianchini et al. 2015). China has seen a five-fold increase in the adoption of conservation agriculture practices between 2008–09 and 2013 (Kassam et al. 2015), which is likely to be linked to the recent emergence of FCR in this region.

F. graminearum has been shown to survive saprophytically on residues of non-host crops such as soybeans, which could be adding to FHB inoculum loads in soybean-wheat rotations. However, *F. graminearum* was recovered to a lesser extent from soybeans than from host crops such as maize and wheat and therefore rotation still provided significant yield and quality advantages under FHB pressure (Dill-Macky and Jones 2000). It would be interesting to determine the saprophytic survival of *Fusarium* species associated with FHB and FCR on other non-host crop species such as chickpeas (*Cicer arietinum*), canola (*Brassica napus*), lentils (*Lens culinaris*) and faba beans (*Vicia faba*), which are commonly grown in rotation with wheat in China, India, Australia and Canada. Limited studies have shown that saprophytic colonisation of non-host species can change the aggressiveness of *F. graminearum* and *F. pseudograminearum* in wheat (generally decreasing it). Pathogenic and saprophytic fitness traits appeared to be independently controlled in both species (Akinsanmi et al. 2004).

Policies and Societal Pressures

The global cropping area is predicted to decrease by 8–20% in the next 30 years, due to land degradation, urban expansion, and an increase in non-food crop production (Nellemann et al. 2009). Non-food cereal production, such as maize for

biofuels in the USA and wheat grain for animal feed to meet increasing demand for meat in developing countries, has been identified as a threat to global food availability, because the same land could be used to grow cereals for direct human consumption (Nellemann et al. 2009). Currently, nearly half of the global cereal production area is used for animal feed, whereas meat accounts for around only 8% of world calorie intake (FAO et al. 2017). Sustainable use of biodiversity and genetic resources has been identified as one of the keys to resolving food security issues worldwide (FAO et al. 2017). However, genetic modification (GM) is not universally regarded as an acceptable tool for crop improvement, particularly in Europe, and this may inhibit valuable developments in plant breeding, including expression of resistance or tolerance traits to FHB or FCR. Limiting these technologies has multiple consequences, from increasing reliance on fungicides to holding back progress in developing countries, which rely on advances made in developed countries to "leapfrog" their own biotechnologies (Yudelman et al. 1998). This is particularly relevant now, as investment in agricultural research has fallen in developing countries since the 1980s (Clapp and Helleiner 2012). Furthermore, GM technologies have been shown to have a two-fold benefit – higher yield and lower chemical inputs – in the case of GM cash crops (Thirtle et al. 2003). Such technologies could be vital to reducing the global impact of *Fusarium* species on wheat production and to meeting future food security targets.

Conclusions

Fusarium species cause regular, seasonal outbreaks of diseases in wheat which result in significant yield loss worldwide. These diseases are exacerbated by extremes in climatic conditions (increased rainfall for FHB and increased drought for FCR), and epidemics of both diseases are likely to increase in different regions under the future pressures of climate change and productivity intensification. Although major achievements have been made in understanding and controlling FHB and FCR, these diseases remain a major limitation to wheat production in some seasons across all wheat-growing regions, and not all regions have access to the risk-reducing technologies available. Thus, to successfully minimise yield loss caused by FHB and FCR on a global scale, an interdisciplinary and collaborative approach is required, especially to improve management in developing countries (e.g. in Asia and Africa) where food insecurity is an ongoing and serious problem. Facilitating scientific exchange between established research groups with extensive FHB or FCR expertise and regions vulnerable to increasing impacts from FCR (e.g. Australia-China) or FHB (e.g. USA-Africa) will be important to fast-track development and adoption of localised integrated disease management strategies, to limit impacts on food security.

Yield loss in wheat caused by FHB and FCR already impacts food security, food safety and grain markets on a global scale. The challenge going forward is to assess the impact of FHB and FCR on food security using a systems approach, not just

focusing on yield loss but also considering grain availability, accessibility, affordability and safety, in line with the framework proposed by Savary et al. (2017). Coordinated information on a global scale would serve as a benchmark for future monitoring of FHB and FCR and their impacts, and as quantitative evidence of the damage inflicted by *Fusarium* species, to guide policy development and future research investment. This would provide the opportunity to manage the current and changing impacts of FHB and FCR, and help producers meet the nutritional needs of a growing global population.

Acknowledgments This review was supported by the Grains Research and Development Corporation (GRDC) (DAN00200), the Australian Government, and the New South Wales Department of Primary Industries (NSW DPI). We thank Professor Ruth Dill-Macky (University of Minnesota) and Keith Pengilley (NSW Department of Primary Industries) for their valuable comments on this chapter.

References

Ablova, I. B., & Slusarenko, A. N. (1997). Problems associated with breeding winter wheat for head scab resistance. In H. J. Dubin, L. Gilchrist, J. Reeves, & A. McNab (Eds.), *Fusarium head scab: Global status and future prospects. Proceedings of a workshop held at CIMMYT,* El Batan, Mexico 1996, pp 93.

Akinsanmi, O. A., Mitter, V., Simpfendorfer, S., Backhouse, D., & Chakraborty, S. (2004). Identity and pathogenicity of *Fusarium* spp. isolated from wheat fields in Queensland and northern New South Wales. *Australian Journal of Agricultural Research, 55*, 97–107.

Alahmad, S., Simpfendorfer, S., Bentley, A., & Hickey, L. (2018). Crown rot of wheat in Australia: *Fusarium pseudograminearum* taxonomy, population biology and disease management. *Australasian Plant Pathology, 47*, 1–15.

Backhouse, D., Abubakar, A. A., Burgess, L. W., Dennis, J. I., Hollaway, G. J., Wildermuth, G. B., Wallwork, H., & Henry, F. J. (2004). Survey of *Fusarium* species associated with crown rot of wheat and barley in eastern Australia. *Australasian Plant Pathology, 33*, 255–261.

Bakker, M. G., Brown, D. W., Kelly, A. C., Kim, H. S., Kurtzman, C. P., McCormick, S. P., O'Donnell, K. L., Proctor, R. H., Vaughan, M. M., & Ward, T. J. (2018). *Fusarium* mycotoxins: A trans-disciplinary overview. *Journal Canadian Journal of Plant Pathology, 40*, 161–171.

Balmas, V., Scherm, B., Marcello, A., Beyer, M., Hoffmann, L., Migheli, Q., & Pasquali, M. (2015). *Fusarium* species and chemotypes associated with *Fusarium* head blight and *Fusarium* root rot on wheat in Sardinia. *Plant Pathology, 64*, 972–979.

Bekele, B. (2018). Review on the status and management strategies of *Fusarium* head blight (*Fusarium graminearum*) of wheat. *The Academic Research Journal of Agricultural Science and Research, 6*, 77–88.

Bentley, A., Cromey, M., Farrokhi-Nejad, R., Leslie, J., Summerell, B., & Burgess, L. (2006). *Fusarium* crown and root rot pathogens associated with wheat and grass stem bases on the South Island of New Zealand. *Australasian Plant Pathology, 35*, 495–502.

Bianchini, A., Horsley, R., Jack, M. M., Kobielush, B., Ryu, D., Tittlemier, S., Wilson, W. W., Abbas, H. K., Abel, S., & Harrison, G. (2015). DON occurrence in grains: A North American perspective. *Cereal Foods World, 60*, 32–56.

Bita, C., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Frontiers in Plant Science, 4*, 273.

Bockus, W. W., Bowden, R., Hunger, R., Murray, T., & Smiley, R. (2010). *Compendium of wheat diseases and pests* (Ed. 3 ed., pp. 34–39). St Paul: American Phytopathological Society (APS Press), The American Phytopathological Society.

Bottalico, A. (1998). *Fusarium* diseases of cereals: species complex and related mycotoxin profiles. *European Journal of Plant Pathology, 80*, 85–103.

Brahim, E. (2012). State of the art of root rot diseases in Morocco. In R. I. S. Brettell & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement* (pp. 12–13). Australia: Narrabri.

Burgess, L., Backhouse, D., Summerell, B. A., & Swan, L. J. (2001). Crown rot of wheat. In B. A. Summerell et al. (Eds.), *Fusarium: Paul E Nelson memorial symposium* (pp. 271–294). St Paul: APS Press, The American Phytopathological Society.

Burgess, L., Dodman, R., Pont, W., & Mayers, P. (1981). *Fusarium diseases of wheat, maize and grain sorghum in eastern Australia. Fusarium: Diseases, biology and taxonomy* (pp. 64–76). University Park: Penn State University Press.

Burgess, L., Wearing, A., & Toussoun, T. (1975). Surveys of Fusaria associated with crown rot of wheat in eastern Australia. *Australian Journal of Agricultural Research, 26*, 791–799.

Carranza, M., Moschini, R. C., Kraan, G., & Bariffi, J. H. (2007). Examination of meteorology-based predictions of *Fusarium* head blight of wheat grown at two locations in the southern Pampas region of Argentina. *Australasian Plant Pathology, 36*, 305–308.

Chakraborty, S., Liu, C. J., Mitter, V., Scott, J. B., Akinsanmi, O. A., Ali, S., Dill-Macky, R., Nicol, J., Backhouse, D., & Simpfendorfer, S. (2006). Pathogen population structure and epidemiology are keys to wheat crown rot and *Fusarium* head blight management. *Australasian Plant Pathology, 35*, 643–655.

Chakraborty, S., & Newton, A. C. (2011). Climate change, plant diseases and food security: An overview. *Plant Pathology, 60*, 2–14.

Chaudhary, R., & Edison, S. (1991). Epidemiology and basic facts of severity, yield loss and grain quality deterioration due to ear blight of wheat in Arunachal Pradesh. *Indian PhytopathologyIndian Phytopathology, 43*, 571–574.

Christensen, J. H., & Christensen, O. B. (2007). A summary of the PRUDENCE model projections of changes in European climate by the end of this century. *Climatic Change, 81*, 7–30.

Clapp, J., & Helleiner, E. (2012). Troubled futures? The global food crisis and the politics of agricultural derivatives regulation. *Review of International Political Economy, 19*, 181–207.

Cook, R. J. (1968). *Fusarium* root and foot rot of cereals in Pacific Northwest. *Phytopathology, 58*, 127.

Cook, R. J. (1980). *Fusarium* foot rot of wheat and its control in the Pacific Northwest. *Plant Disease, 64*, 1061–1066.

Cowger, C., Sutton, A. L. (2005). The southeastern U.S. *Fusarium* head blight epidemic of 2003. Online. *Plant Health Progress.* https://doi.org/10.1094/PHP-2005-1026-01-RS

Cromey, M., Shorter, S., Lauren, D., & Sinclair, K. (2002). Cultivar and crop management influences on *Fusarium* head blight and mycotoxins in spring wheat (*Triticum aestivum*) in New Zealand. *New Zealand Journal of Crop and Horticultural Science, 30*, 235–247.

Curtis, T., & Halford, N. (2014). Food security: The challenge of increasing wheat yield and the importance of not compromising food safety. *The Annals of Applied Biology, 164*, 354–372.

Dahl, B., & Wilson, W. W. (2018). Risk premiums due to *Fusarium* head blight (FHB) in wheat and barley. *Agricultural Systems, 162*, 145–153.

de Galich, M. T. V. (1997). *Fusarium* head blight in Argentina. In H. J. Dubin, L Gilchrist, J. Reeves & A. McNab (Eds.), *Fusarium head scab: Global status and future prospects. Proceedings of a Workshop Held at CIMMYT,* El Batan, Mexico 1996, pp. 19–28.

de Villiers, C. I. (2014). Glasshouse screening of CIMMYT wheat germplasm for *Fusarium* head blight response in South Africa. *South African Journal of Plant and Soil, 31*, 49–51.

De Wolf, E., Madden, L., & Lipps, P. (2003). Risk assessment models for wheat *Fusarium* head blight epidemics based on within-season weather data. *Phytopathology, 93*, 428–435.

Del Ponte, E. M., Fernandes, J. M. C., & Pavan, W. (2005). A risk infection simulation model for *Fusarium* head blight of wheat. *Fitopatologia Brasileira, 30*, 634–642.

Diaz de Ackermann, M., Kohli, M. M. (1997). Research on *Fusarium* head blight of wheat in Uruguay. In H. J. Dubin, L. Gilchrist, J. Reeves, & A. McNab, (Eds.), *Fusarium head scab: Global status and future prospects. Proceedings of a Workshop Held at CIMMYT*, El Batan, Mexico 1996, pp. 13–18.

Dill-Macky, R., & Jones, R. (2000). The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. *Plant Disease, 84*, 71–76.

Duba, A., Goriewa-Duba, K., & Wachowska, U. (2018). A review of the interactions between wheat and wheat pathogens: *Zymoseptoria tritici*, *Fusarium* spp. and *Parastagonospora nodorum*. *International Journal of Molecular Sciences, 19*, 1138.

Dubin, H., & Bimb, H. (1994). Soil borne diseases of wheat in warmer areas of South Asia: An update. In D. Saunders & G. P. Hettel (Eds.), *Wheat in heat stressed environments: Irrigated, dry areas and rice wheat farming systems* (pp. 353–360). Mexico: UNDP/ARC/BARI/CIMMYT.

Fakhfakh, M., Yahyaoui, A., Rezgui, S., Elias, E., & Daaloul, A. (2011). Identification and pathogenicity assessment of *Fusarium* spp. sampled from durum wheat fields in Tunisia. *African Journal of Biotechnology, 10*, 6529–6539.

Food and Agriculture Organization (FAO). (2018). Agricultural Management Information System Statistics (AMIS) Market Database. http://statistics.amis-outlook.org/data/index.html. Accessed 30 Aug 2018.

FAO, IFAD, UNICEF, WFP and WHO. (2017). The state of food security and nutrition in the world 2017. Building resilience for peace and food security. http://www.fao.org/3/a-I7695e.pdf. Accessed 29 Sept 2018.

Fernandez, M., & Chen, Y. (2005). Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. *Plant Disease, 89*, 164–169.

Fernandez, M., & Zentner, R. (2005). The impact of crop rotation and N fertilizer on common root rot of spring wheat in the brown soil zone of western Canada. *Canadian Journal of Plant Science, 85*, 569–575.

Food Security Information Network (FSIN). (2018) Global report on food crises 2018. https://www.wfp.org/content/global-report-food-crises-2018. Accessed 24 Sept 2018.

Gale, L. R., Chen, L.-F., Hernick, C., Takamura, K., & Kistler, H. (2002). Population analysis of *Fusarium graminearum* from wheat fields in eastern China. *Phytopathology, 92*, 1315–1322.

Gargouri, S., Berraies, S., Gharbi, S., Chekali, S., Fakhfakh, M., Burgess, L. W. (2012). Tunisia: An overview of *Fusarium* diseases in wheat and the unique farming systems of the region. In R. I. S. Brettell, & J. M. Nicol, (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement,* Narrabri, Australia, pp. 15.

Ghodbane, A., Mahjoub, M., Djerbi, M., Mlaiki, A., & Sharen, A. (1974). Étude des pertes causées par les pathogènes du blé, *Septoria tritici* et *Fusarium* spp Rapport annuel du ministère de l'agriculture Office des Céréales, Tunisie, pp. 106.

Godfray, H. C., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Sciencexpress Review, 28*, 1185383.

Goswami, R., & Kistler, C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. *Molecular Plant Pathology, 5*(6), 515–525.

Haidukowski, M., Visconti, A., Perrone, G., Vanadia, S., Pancaldi, D., Covarelli, L., Balestrazzi, R., & Pascale, M. (2012). Effect of prothioconazole-based fungicides on *Fusarium* head blight, grain yield and deoxynivalenol accumulation in wheat under field conditions. *Phytopathologia Mediterranea, 51*, 236–246.

Hameed, M., Rana, R., & Ali, Z. (2012). Identification and characterization of a novel Iraqi isolate of *Fusarium pseudograminearum* causing crown rot in wheat. *Genetics and Molecular Research, 11*, 1341–1348.

Hanjra, M. A., & Qureshi, M. E. (2010). Global water crisis and future food security in an era of climate change. *Food Policy, 35*, 365–377.

Hekimhan, H., Bagci, A., Nicol, J., Arisoy, Z., Taner, S., & Sahin, S. (2004). Poster: Dryland root rot: A major threat to winter cereal production under sub-optimal growing conditions. In *New directions for a diverse planet. Proceedings for the 4th International Crop Science Congress*, Brisbane, Australia.

Hollaway, G. J., Evans, M. L., Wallwork, H., Dyson, C. B., & McKay, A. C. (2013). Yield loss in cereals, Caused by *Fusarium culmorum* and *F. pseudograminearum*, is related to fungal DNA in soil prior to planting, rainfall, and cereal type. *Plant Disease, 97*, 977–982.

Hollaway, G. J., Excell, G. K. (2012). A survey of crown rot in Victoria wheat crops: 1997–2009. In R. I. S. Brettell, & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement*, Narrabri, Australia, pp 21.

Huang, Y., & Wong, P. (1998). Effect of *Burkholderia (Pseudomonas)cepacia* and soil type on the control of crown rot in wheat. *Plant and Soil, 203*, 103–108.

Hüberli, D., Gajda, K., Connor, M., & Van Burgel, A. (2017). Choosing the best yielding wheat and barley variety under high crown rot. In *2017 Grains Research Updates*, Perth, Western Australia, 27–28 February. http://researchrepository.murdoch.edu.au/id/eprint/35682/

Imathiu, S. M., Ray, R. V., Back, M. I., Hare, M. C., & Edwards, S. G. (2013). A survey investigating the infection of *Fusarium langsethiae* and production of HT-2 and T-2 mycotoxins in UK oat fields. *Journal of Phytopathology, 161*, 553–561.

Ioos, R., Belhadj, A., & Menez, M. (2004). Occurrence and distribution of *Microdochium nivale* and *Fusarium* species isolated from barley, durum and soft wheat grains in France from 2000 to 2002. *Mycopathologia, 158*, 351.

Iram, S., Ashraf, M., & Ahmad, I. (2003). Prevalence, incidence and severity of soil-borne diseases and fungi of wheat in rice-wheat cropping system of Punjab Province of Pakistan during the cropping season 1999. *Pakistan Journal of Biological Sciences, 6*, 36–40.

Ji, L., Kong, L., Li, Q., Wang, L., Chen, D., & Ma, P. (2016). First report of *Fusarium pseudograminearum* causing *Fusarium* head blight of wheat in Hebei Province, China. *Plant Disease, 100*, 220.

Ji, L., Kong, L., Wang, Y., & Li, Q. (2018). Occurrence and development of wheat crown rot in Hebei province, China. In *10th ASDS Proceedings, September 2018,* Adelaide, pp. 1–2.

Karlsson, I., Friberg, H., Kolseth, A.-K., Steinberg, C., & Persson, P. (2017). Agricultural factors affecting *Fusarium* communities in wheat kernels. *International Journal of Food Microbiology, 252*, 53–60.

Kassam, A., Friedrich, T., Derpsch, R., & Kienzle, J. (2015). Overview of the worldwide spread of conservation agriculture. In *Field Actions Science Reports*. http://factsreports.revues.org/3966 Accessed 28 Sept 2018.

Kazan, K., & Gardiner, D. M. (2018). *Fusarium* crown rot caused by *Fusarium pseudograminearum* in cereal crops: Recent progress and future prospects. *Molecular Plant Pathology, 19*, 1547–1562.

Kephart, K. (1991). Climatic conditions and regional differences. In E. Jones (Ed.), *Soft red winter wheat quality: Issues for producers, merchants, and millers* (pp. 5–10). Blacksburg: Virginia Polytechnic Institute.

Khangura, R. K., MacNish, G. C., MacLeod, W. J., Vanstone, V. A., Hanbury, C. D., Loughman, R., & Speijers, J. E. (2013). Current status of cereal root diseases in Western Australia under intensive cereal production and their comparison with the historical survey conducted during 1976–1982. *Journal of Phytopathology, 161*, 828–840.

Klaasen, J., Matthee, F., Marasas, W., & Van Schalkwyk, D. (1992). Survey of *Fusarium* species associated with crowns of healthy-head and white-head wheat plants in the southern and western Cape Province. *Phytophylactica, 24*, 85–94.

Klein, T. A., Burgess, L. W., & Ellison, F. W. (1991). The incidence and spatial patterns of wheat plants infected by *Fusarium graminearum* group 1 and the effect of crown rot on yield. *Australian Journal of Agricultural Research, 42*, 399–407.

Knight, N. L., MacDonald, B., & Sutherland, M. W. (2017). Colonization of durum wheat (*Triticum turgidum* L. var. durum) culms exhibiting premature senescence (dead heads) associated with *Fusarium pseudograminearum* crown rot. *Plant Disease, 101*, 1788–1794.

Kriel, W., & Pretorius, Z. (2008). The FHB challenge to irrigation wheat production in South Africa. *Cereal Research Communications, 36*, 569–571.

Lakhesar, D. P. S., Backhouse, D., & Kristiansen, P. (2010). Nutritional constraints on displacement of *Fusarium* pseudograminearum from cereal straw by antagonists. *Biological Control, 55*, 241–247.

Lang, T. (2010). Crisis? What crisis? The normality of the current food crisis. *Journal of Agrarian Change, 10*, 87–97.

Lauren, D. R., Sayer, S. T., & di Menna, M. E. (1992). Trichothecene production by *Fusarium* species isolated from grain and pasture throughout New Zealand. *Mycopathologia, 120*, 167–176.

Li, H. B., Xie, G. Q., Ma, J., Liu, G. R., Wen, S. M., Ban, T., Chakraborty, S., & Liu, C. J. (2010). Genetic relationships between resistances to *Fusarium* head blight and crown rot in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics, 121*, 941–950.

Li, H. L., Yuan, H. X., Fu, B., Xing, X. P., Sun, B. J., & Tang, W. H. (2012). First report of *Fusarium pseudograminearum* causing crown rot of wheat in Henan, China. *Plant Disease, 96*, 1065–1065.

Li, X., Shin, S., Heinen, S., Dill-Macky, R., Berthiller, F., Nersesian, N., Clemente, T., McCormick, S., & Muehlbauer, G. J. (2015). Transgenic wheat expressing a barley UDP-glucosyltransferase detoxifies deoxynivalenol and provides high levels of resistance to *Fusarium graminearum*. *Molecular Plant-Microbe Interactions, 28*, 1237–1246.

Lilliboe, D. (Ed.). (2008). Déjà vu – '08 scab situation similar to '07 *Fusarium*. *Focus, 8*, 2. http://www.scabusa.org/pdfs/fus-focus_10-08_newsletter.pdf. Accessed 28 Sept 2018.

Lindblad, M., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olsen, M., & Fredlund, E. (2013). Deoxynivalenol and other selected *Fusarium* toxins in Swedish wheat: Occurrence and correlation to specific *Fusarium* species. *International Journal of Food Microbiology, 167*, 284–291.

Ma, J., Li, H. B., Zhang, C. Y., Yang, X. M., Liu, Y. X., Yan, G. J., & Liu, C. J. (2010). Identification and validation of a major QTL conferring crown rot resistance in hexaploid wheat. *Theoretical and Applied Genetics, 120*, 1119–1128.

Mains E, Curtis P (1929) Scab of small grains and feeding trouble in Indiana in 1928. In *Proceedings of the Indiana Academy of Science*, pp 101–110.

Martin, R. A., & Johnston, H. W. (1982). Effects and control of *Fusarium* diseases of cereal grains in the Atlantic Provinces. *Canadian Journal of Plant Pathology, 4*, 210–216.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. *Plant Disease, 96*, 1712–1728.

McMullen, M., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: A re-emerging disease of devastating impact. *Plant Disease, 81*, 1340–1348.

Melloy, P., Hollaway, G., Luck, J., Norton, R., Aitken, E., & Chakraborty, S. (2010). Production and fitness of *Fusarium pseudograminearum* inoculum at elevated carbon dioxide in FACE. *Global Change Biology, 16*, 3363–3373.

Mergoum, M., Nsarellah, N., & Nachit, M. (1997). Evaluation of durum wheat germplasm resistance to root and foot rot disease complex (*Fusarium culmorum* and *Cochliobolus sativus*) in Morocco. *Plant Genetic Resources Newsletter, 109*, 11–14.

Miedaner, T., Gwiazdowska, D., & Waskiewicz, A. (2017). Editorial: Management of *Fusarium* Species and their mycotoxins in cereal food and feed. *Frontiers in Microbiology, 8*, 3.

Miller, J. D. (2008). Mycotoxins in small grains and maize: old problems, new challenges. *Food Additives and Contaminants, 25*, 219–230.

Minnaar-Ontong, A., Herselman, L., Kriel, W.-M., & Leslie, J. F. (2017). Morphological characterization and trichothecene genotype analysis of a *Fusarium* head blight population in South Africa. *European Journal of Plant Pathology, 148*, 261–269.

Moya-Elizondo, E., Arismendi, N., Castro, M. P., & Doussoulin, H. (2015). Distribution and prevalence of crown rot pathogens affecting wheat crops in southern Chile. *Chilean Journal of Agricultural Research, 75*, 78–84.

Moya-Elizondo, E. A., Rew, L. J., Jacobsen, B. J., Hogg, A. C., & Dyer, A. T. (2011). Distribution and prevalence of *Fusarium* crown rot and common root rot pathogens of wheat in Montana. *Plant Disease, 95*, 1099–1108.

Mudge, A. M., Dill-Macky, R., Dong, Y., Gardiner, D. M., White, R. G., & Manners, J. M. (2006). A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*. *Physiological and Molecular Plant Pathology, 69*, 73–85.

Murray, G. M., & Brennan, J. P. (2009). Estimating disease losses to the Australian wheat industry. *Australasian Plant Pathology, 38*, 558–570.

Muthomi, J., Riungu, G., Ndung'u, J., & Narla, R. (2007). Occurrence of wheat head blight and *Fusarium* species infecting wheat. *African Crop Science Conference Proceedings, 8*, 863–867.

Nellemann, C., MacDevette, M., Manders, T., Eickhout, B., Svihus, B., Prins, A. G., & Kaltenborn, B. P. (Eds.). (2009). *The environmental food crisis: the environment's role in averting future food crises: A UNEP rapid response assessment* (pp. 11–33). Birkeland Trykkeri AS: United Nations Environment Programme.

Nganje, W. E., Bangsund, D. A., Leistritz, F. L., Wilson, W. W., & Tiapo, N. M. (2004). Regional economic impacts of *Fusarium* head blight in wheat and barley. *Review of Agricultural Economics, 26*, 332–347.

Nicol, J., Dababat, A. A., Erginbas, G., Bolat, N., Yorgincilar, A., Tunali, B., Bagci, S. A., Hekimham, H., Ozdemir, F., Morgounov, A., Manes, Y., Singh, R. P., Braun, H. J., & Trethowan, R. M. (2012). An international perspective on CIMMYT-Turkey research on *Fusarium* crown rot of wheat – progress and future perspectives with reference to Australian germplasm improvement. In R. I. S. Brettell & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement* (pp. 7–9). Australia: Narrabri.

Nielsen, L. K., Jensen, J. D., Nielsen, G. C., Jensen, J. E., Spliid, N. H., Thomsen, I. K., Justesen, A. F., Collinge, D. B., & Jørgensen, L. N. (2011). *Fusarium* head blight of cereals in Denmark: species complex and related mycotoxins. *Phytopathology, 101*, 960–969.

Obanor, F., Neate, S., Simpfendorfer, S., Sabburg, R., Wilson, P., & Chakraborty, S. (2013). *Fusarium graminearum* and *Fusarium pseudograminearum* caused the 2010 head blight epidemics in Australia. *Plant Pathology, 62*, 79–91.

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144*, 31–43.

Oerke, E. C., Dehne, H.-W., Schönbeck, F., & Weber, A. (2012). *Crop production and crop protection: estimated losses in major food and cash crops* (pp. 272–273). Amsterdam: Elsevier.

Pancaldi, D., Tonti, S., Prodi, A., Salomoni, D., Dal Prà, M., Nipoti, P., Alberti, I., & Pisi, A. (2010). Survey of the main causal agents of *Fusarium* head blight of durum wheat around Bologna, Northern Italy. *Phytopathologia Mediterranea, 49*, 258–266.

Parikka, P., Hakala, K., & Tiilikkala, K. (2012). Expected shifts in *Fusarium* species' composition on cereal grain in Northern Europe due to climatic change. *Food Additives & Contaminants: Part A, 29*, 1543–1555.

Parry, D., Jenkinson, P., & McLeod, L. (1995). *Fusarium* ear blight (scab) in small grain cereals—a review. *Plant Pathology, 44*, 207–238.

Pasquali, M., Beyer, M., Logrieco, A., Audenaert, K., Balmas, V., Basler, R., Boutigny, A.-L., Chrpová, J., Czembor, E., Gagkaeva, T., González-Jaén, M. T., Hofgaard, I. S., Köycü, N. D., Hoffmann, L., Lević, J., Marin, P., Miedaner, T., Migheli, Q., Moretti, A., Müller, M. E. H., Munaut, F., Parikka, P., Pallez-Barthel, M., Piec, J., Scauflaire, J., Scherm, B., Stanković, S., Thrane, U., Uhlig, S., Vanheule, A., Yli-Mattila, T., & Vogelgsang, S. (2016). A European database of *Fusarium graminearum* and *F. culmorum* trichothecene genotypes. *Frontiers in Microbiology, 7*, 406.

Paul, P., Lipps, P., Hershman, D., McMullen, M., Draper, M., & Madden, L. (2008). Efficacy of triazole-based fungicides for *Fusarium* head blight and deoxynivalenol control in wheat: A multivariate meta-analysis. *Phytopathology, 98*, 999–1011.

Paulitz, T. C., Smiley, R. W., & Cook, R. J. (2002). Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. *Canadian Journal of Plant Pathology, 24*, 416–428.

Pereyra, S., Dill-Macky, R., & Sims, A. (2004). Survival and inoculum production of *Gibberella zeae* in wheat residue. *Plant Disease, 88*, 724–730.

Petronaitis, T., Forknall, C., & Simpfendorfer, S. (2018). Microwave radiation reduces survival of *Fusarium pseudograminearum* in durum wheat stubble. *Australasian Plant Pathology, 47*, 375–378.

Pineiro, M. (1997). *Fusarium* toxins in Uruguayan wheat. In H. J. Dubin, L. Gilchrist, J. Reeves, & A. McNab (Eds.), *Fusarium head scab: Global status and future prospects. Proceedings of a Workshop Held at CIMMYT*, El Batan, Mexico 1996, pp. 126.

Pirgozliev, S., Ray, R., Edwards, S., Hare, M., & Jenkinson, P. (2008). Effect of timing of fungicide application on the development of *Fusarium* head blight and the accumulation of deoxynivalenol (DON) in winter wheat grain. *Cereal Research Communications, 36*, 289–299.

Pitt, J. I., & Miller, J. D. (2017). A concise history of mycotoxin research. *Journal of Agricultural and Food Chemistry, 65*, 7021–7033.

Poole, G. J., Smiley, R. W., Walker, C. A., Garland-Campbell, K., & Paulitz, T. C. (2012). Distribution of *Fusarium* spp. causing crown rot in the Pacific North West. In R. I. S. Brettell & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement* (p. 22). Australia: Narrabri.

Pouzeshimiab, B., Razavi, M., Zare, R., Zamanizadeh, H. R., Rezaee, S., Safaee, D., & Nicol, J. (2012). Taxonomy and distribution of *Fusarium* spp. associated with root and crown rot of wheat in Iran. In R. I. S. Brettell, & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement*, Narrabri, Australia, p. 31.

Proctor, R. H., Hohn, T. M., & McCormick, S. P. (1995). Reduced virulence of *Gibberella zeae* caused by disruption of a trichthecine toxin biosynthetic gene. *Molecular Plant-Microbe Interactions, 8*, 593–601.

Sabburg, R., Obanor, F., Aitken, E., & Chakraborty, S. (2015). Changing fitness of a necrotrophic plant pathogen under increasing temperature. *Global Change Biology, 21*, 3126–3137.

Safaee, D., Razavi, M., Mahdavi, M., Younesi, H., & Nicol, J. (2012). The current situation of crown and foot rot diseases of wheat in Iran; what has been done and what should be done. In R. I. S. Brettell & J. M. Nicol (Eds.), *Proceedings of the first international crown rot workshop for wheat improvement* (p. 6). Australia: Narrabri.

Saremi, H., Ammarellou, A., & Jafary, H. (2007). Incidence of crown rot disease of wheat caused by *Fusarium pseudograminearum* as a new soil born fungal species in North West Iran. *Pakistan Journal of Biological Sciences, 10*, 3606–3612.

Savary, S., Bregaglio, S., Willocquet, L., Gustafson, D., Mason D'Croz, D., Sparks, A., Castilla, N., Djurle, A., Allinne, C., Sharma, M., Rossi, V., Amorim, L., Bergamin, A., Yuen, J., Esker, P., McRoberts, N., Avelino, J., Duveiller, E., Koo, J., & Garrett, K. (2017). Crop health and its global impacts on the components of food security. *Food Security, 9*, 311–327.

Sayer, S., & Lauren, D. (1991). *Fusarium* infection in New Zealand grain. *New Zealand Journal of Crop and Horticultural Science, 19*, 143–148.

Schaafsma, A., & Hooker, D. (2007). Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. *International Journal of Food Microbiology, 119*, 116–125.

Schmale, D., Wood-Jones, A., Cowger, C., Bergstrom, G., & Arellano, C. (2011). Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. *Plant Pathology, 60*, 909–917.

Schoneberg, T., Jenny, E., Wettstein, F. E., Bucheli, T. D., Mascher, F., Bertossa, M., Musa, T., Seifert, K., Grafenhan, T., Keller, B., & Vogelgsang, S. (2018). Occurrence of *Fusarium* species

and mycotoxins in Swiss oats – impact of cropping factors. *European Journal of Agronomy, 92*, 123–132.

Scott, P., & de Jager, D. (1988). *Fusarium* blight of irrigated wheat in South Africa. *Phytophylactica, 20*, 317–320.

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security, 4*, 178–202.

Shah, N., & Paulsen, G. (2003). Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and Soil, 257*, 219–226.

Simpfendorfer, S. (2016a). *Crown rot – Does cereal crop or variety choice matter?* GRDC Update Paper, February 2016. https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2016/02/crown-rot-does-cereal-crop-or-variety-choice-matter. Accessed 25 Sept 2018.

Simpfendorfer, S. (2016b). Evaluation of fungicide timing and preventative application on the control of *Fusarium* head blight and resulting yield. *Australasian Plant Pathology, 45*, 513–516.

Simpfendorfer, S. (2016c). *Evaluation of the seed treatment Rancona Dimension as a standalone option for managing crown rot in wheat – 2015*. NSW DPI Northern Grains Research Results, Tamworth, Australia, p 121.

Simpfendorfer, S., & Gardner, M. (2013). *Crown rot: Be aware of the balancing act or the fall may be harder*. https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2013/02/crown-rot-be-aware-of-the-balancing-act-or-the-fall-may-be-harder. Accessed 28 Sept 2018.

Simpfendorfer, S., Giblot-Ducray, D., Hartley, D., & McKay, A. (2017). *Where did the low levels of Fusarium head blight come from in 2016 and what does it mean?*. https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2017/02/where-did-the-low-levels-of-fusarium-head-blight-come-from-in-2016-and-what-does-it-mean. Accessed 28 Sept 2018.

Smiley, R. W., Collins, H. P., & Rasmussen, P. E. (1996). Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. *Plant Disease, 80*, 813–820.

Smiley, R. W., Gourlie, J. A., Easley, S. A., Patterson, L. M., & Whittaker, R. G. (2005). Crop damage estimates for crown rot of wheat and barley in the Pacific Northwest. *Plant Disease, 89*, 595–604.

Southwell, R., Moore, K., Manning, W., & Hayman, P. (2003). An outbreak of *Fusarium* head blight of durum wheat on the Liverpool Plains in northern New South Wales in 1999. *Australasian Plant Pathology, 32*, 465–471.

Stewart, J. (2003). Feature: The World Food Programme's response to the Southern African humanitarian crisis: Moving food. *African Security Review, 12*, 17–27.

Strange, R. N., & Scott, P. R. (2005). Plant disease: A threat to global food security. *Annual Review of Phytopathology, 43*, 83–116.

Summerell, B. A., & Burgess, L. W. (1988). Stubble management practices and the survival of *Fusarium graminearum* Group 1 in wheat stubble residues. *Australasian Plant Pathology, 17*, 88–93.

Sydenham, S., & de Villiers, C. (2014). *Reducing the risk of Fusarium head blight disease for irrigated wheat*. https://www.grainsa.co.za/reducing-the-risk-of-fusarium-head-blight-disease-for-irrigated-wheat. Accessed 27 Sept 2018.

Tan, M. K., Simpfendorfer, S., Backhouse, D., & Murray, G. M. (2004). Occurrence of *Fusarium* head blight (FHB) in southern NSW in 2000: Identification of causal fungi and determination of putative chemotype of *Fusarium graminearum* isolates by PCR. *Australasian Plant Pathology, 33*, 385–392.

Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U. S., Sugiura, Y., & Ueno, Y. (1988). Worldwide contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and zearalenone. *Journal of Agricultural and Food Chemistry, 36*, 979–983.

Teli, B., Chattopadhyay, A., Meena, S. C., Gangwar, G. P., & Pandey, S. K. (2016). Present status of *Fusarium* head blight of wheat and barley in India. *Diseases of Wheat and Their Management, 2016*, 79–92.

Thirtle, C., Beyers, L., Ismael, Y., & Piesse, J. (2003). Can GM-technologies help the poor? The impact of Bt cotton in Makhathini Flats, KwaZulu-Natal. *World Development, 31*, 717–732.

Tralamazza, S. M., Braghini, R., & Corrêa, B. (2016). Trichothecene Genotypes of the *Fusarium graminearum* species complex isolated from Brazilian wheat grains by conventional and quantitative PCR. *Frontiers in Microbiology, 7*, 246.

Tunali, B., Nicol, J. M., Hodson, D., Uçkun, Z., Büyük, O., Erdurmuş, D., Hekimhan, H., Aktaş, H., Akbudak, M. A., & Bağci, S. A. (2008). Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. *Plant Disease, 92*, 1299–1306.

Tușa, C., Munteanu, I., Capetti, E., Pirvu, T., Bunescu, S., Sin, G., Nicolae, H., Tianu, A., Caea, D., & Romașcanu, O. (1981). Aspects of the *Fusarium* attacks on wheat in Romania. *Probleme de Protecția Plantelor, 9*, 15–31.

United Nations Department of Economic and Social Affairs (UN DESA). (2017). *World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100*. https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html. Accessed 25 Sept 2018.

United States Agency for International Development (USAID). (2018). *Feed the future*. https://www.usaid.gov/what-we-do/agriculture-and-food-security/increasing-food-security-through-feed-future. Accessed 17 Sept 2018.

United States Department of Agriculture (USDA) (2014). *Grain and Feed Annual 2014/2015 Grains Forecast*. https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Grain%20and%20Feed%20Annual_Brasilia_Brazil_3-21-2014.pdf. Accessed 27 Sept 2018.

van der Lee, T., Zhang, H., van Diepeningen, A., & Waalwijk, C. (2015). Biogeography of *Fusarium graminearum* species complex and chemotypes: A review. *Food Additives & Contaminants: Part A, 32*, 453–460.

Varga, E., Wiesenberger, G., Hametner, C., Ward, T. J., Dong, Y., Schöfbeck, D., McCormick, S., Broz, K., Stückler, R., & Schuhmacher, R. (2015). New tricks of an old enemy: Isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. *Environmental Microbiology, 17*, 2588–2600.

Vaughan, M., Backhouse, D., & Ponte, E. D. (2016). Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to *Fusarium* head blight: A review. *World Mycotoxin Journal, 9*, 685–700.

Vermeulen, S. J., Campbell, B. M., & Ingram, J. S. (2012). Climate change and food systems. *Annual Review of Environment and Resources, 37*, 195–222.

Verrell, A. G., Simpfendorfer, S., & Moore, K. J. (2017). Effect of row placement, stubble management and ground engaging tool on crown rot and grain yield in a no-till continuous wheat sequence. *Soil and Tillage Research, 165*, 16–22.

Vrabcheva, T., Geßler, R., Usleber, E., & Märtlbauer, E. (1996). First survey on the natural occurrence of *Fusarium* mycotoxins in Bulgarian wheat. *Mycopathologia, 136*, 47–52.

Walder, F., Schlaeppi, K., Wittwer, R., Held, A. Y., Vogelgsang, S., & van der Heijden, M. G. A. (2017). Community profiling of *Fusarium* in combination with other plant-associated fungi in different crop species using SMRT sequencing. *Frontiers in Plant Science, 8*, 17.

Wallwork, H. (Ed.). (2000). *Cereal root and crown diseases* (p. 58). Kingston: Grains Research and Development Corporation.

Wang, Y. Z. (1997). Epidemiology and management of wheat scab in China. In H. J. Dubin, L. Gilchrist, J. Reeves, & A. McNab (Eds.), *Fusarium head scab: Global status and future prospects. Proceedings of a Workshop Held at CIMMYT*, El Batan, Mexico 1996, pp. 97–105.

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D. E., Gilbert, J., Geiser, D. M., & Nowicki, T. W. (2008). An adaptive evolutionary shift in *Fusarium* head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genetics and Biology, 45*, 473–484.

Wildermuth, G. B., Thomas, G. A., Radford, B. J., McNamara, R. B., & Kelly, A. (1997). Crown rot and common root rot in wheat grown under different tillage and stubble treatments in southern Queensland, Australia. *Soil and Tillage Research, 44*, 211–224.

Windels, C. E. (2000). Economic and social impacts of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. *Phytopathology, 90*, 17–21.

Wu, F., Groopman, J. D., & Pestka, J. J. (2014). Public health impacts of foodborne mycotoxins. *Annual Review of Food Science and Technology, 5*, 351–372.

Xu, F., Song, Y., Yang, G., Wang, J., Liu, L., & Li, Y. (2015). First Report of *Fusarium pseudograminearum* from wheat heads with *Fusarium* head blight in North China Plain. *Plant Disease, 99*, 156–156.

Xu, F., Yang, G., Wang, J., Song, Y., Liu, L., Zhao, K., Li, Y., & Han, Z. (2018). Spatial distribution of root and crown rot fungi associated with winter wheat in the North China Plain and its relationship with climate variables. *Frontiers in Microbiology, 9*, 1054.

Yao, J., Zhou, M., Zhang, X., Ren, L., Yu, G., & Lu, W. (2008). Molecular breeding for wheat *Fusarium* head blight resistance in China. In *3rd International FHB Symposium*, Szeged, Hungary, pp. 203–212.

Yoshida, M., Nakajima, T., Tomimura, K., Suzuki, F., Arai, M., & Miyasaka, A. (2012). Effect of the timing of fungicide application on *Fusarium* head blight and mycotoxin contamination in wheat. *Plant Disease, 96*, 845–851.

Yudelman, M., Ratta, A., & Nygaard, D. F. (1998). *Pest management and food production: Looking to the future* (Vol. 25, p. 33). Washington, DC: The International Food Policy Research Institute.

Zhuping, Y. (1994). Breeding for resistance to *Fusarium* head blight of wheat in the mid-to lower Yangtze River Valley of China. *CIMMYT Wheat Special Report, 27*, 1.

Zhang, X., Zwiers, F. W., Hegerl, G. C., Lambert, F. H., Gillett, N. P., Solomon, S., Stott, P. A., & Nozawa, T. (2007). Detection of human influence on twentieth-century precipitation trends. *Nature, 448*, 461–466.

Chapter 8
Quantitative Assessment of Consequences of Quarantine Plant Pathogen Introductions: From Crop Losses to Environmental Impact

Michael Jeger, Giuseppe Stancanelli, Gianni Gilioli, Gregor Urek, Ariena van Bruggen, Jean-Claude Grégoire, Vittorio Rossi, Wopke van der Werf, Alan MacLeod, Gritta Schader, Sybren Vos, Svetla Kozelska, Marco Pautasso, Ciro Gardi, and Olaf-Mosbach-Schulz

Abstract Pest risk assessments are required to take phytosanitary measures to protect plant health. In the European Union, the Scientific Panel on Plant Health (PLH) of the European Food Safety Authority was established in 2006 as the reference body for risk assessment in the plant health area. Risk assessments address four steps: introduction, establishment, spread and consequences. Until recently, the PLH Panel had made risk assessments requested by the European Commission using qualitative approaches which typically gave risk and associated uncertainty ratings based on ordinal scales. The PLH Panel has now developed guidance on a methodology for quantitative assessment of these four stages. In this chapter, we outline key features of this methodology when applied to assessment of consequences,

M. Jeger (✉)
Centre for Environmental Policy, Imperial College London, Ascot, UK
e-mail: m.jeger@imperial.ac.uk

G. Stancanelli · S. Vos · S. Kozelska · M. Pautasso · C. Gardi
Animal and Plant Health Unit, European Food Safety Authority, Parma, Italy

G. Gilioli
Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

G. Urek
Plant Protection Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia

A. van Bruggen
Department of Plant Pathology and Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA

J.-C. Grégoire
Spatial Epidemiology, Université libre de Bruxelles, Bruxelles, Belgium

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_8

including both crop and environmental impacts, of the introduction and spread of quarantine plant pathogens into new territories. The methodology is then applied to four case studies *Diaporthe vaccinii, Cryphonectria parasitica*, *Ditylenchus destructor*, and the Flavescence dorée phytoplasma – plant pathogens representing a range of taxa, host types and cropping systems.

Introduction

Introductions of plant pests and pathogens to new regions, countries and continents have a range of consequences, especially where disease outbreaks have the potential to develop into major epidemics in time and space. The consequences are multidimensional, even without taking into account socio-economic and cultural considerations (Ghelardini et al. 2017; Gilardi et al. 2018; Jones 2018; Sicard et al. 2018; Suffert et al. 2018) (Table 8.1). The consequences include: crop losses in terms of yield, quality, production area and food security; the need for additional control measures or risk-reducing measures, including long-term changes in the use of plant protection chemicals; impact on amenity, woodland and forest trees; environmental and other undesired impacts of control measures; and impacts on native plants, biodiversity and ecosystem services (Oerke 2006; Savary et al. 2012; Freer-Smith and Webber 2017; Seidl et al. 2018).

There is an increasing use of quantitative methods to assess consequences of plant disease outbreaks (e.g. Donatelli et al. 2017; Rimbaud et al. 2018; Savary et al. 2018) but these are not directed specifically to cases of new introductions of pathogens that are subject to plant health regulation. To this end, new quantitative methods have been developed by the European Food Safety Authority (EFSA Plant Health Panel et al. 2018) to assess the risks of introduction (entry and establishment), and subsequent spread of invasive quarantine plant pests in the European Union (Fig. 8.1), many of which are plant pathogens. The EFSA methodology is based upon a quantitative population-based approach where at each stage of the

V. Rossi
Department of Sustainable Crop Production – DI.PRO.VE.S,
Università Cattolica del Sacro Cuore, Piacenza, Italy

W. van der Werf
Centre for Crop Systems Analysis, Wageningen University, Wageningen, The Netherlands

A. MacLeod
Department for environment, food and rural affairs, York, UK

G. Schader
Julius Kühn-Institut, Braunschweig, Germany

Olaf-Mosbach-Schulz
Assessment and Methodological Support Unit, European Food Safety Authority, Parma, Italy

Table 8.1 Multidimensional consequences of plant disease introduction

Crop losses in terms of yield, quality, and production area
Additional control measures, and long-term increases of the use of plant protection products
Impact on amenity trees, woodlands and forests
Impact on the spread and impact of other pathogens
Environmental and other undesired impacts of control measures
Impacts on native plants, biodiversity and ecosystem services

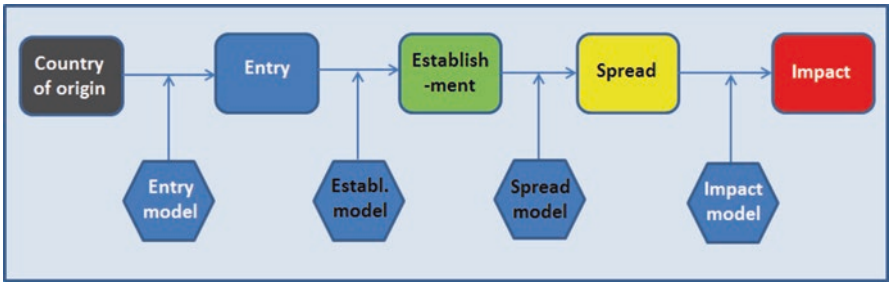

Fig. 8.1 Flow of events in the quantitative modelling approach to risk assessment

assessment, and for each of the estimations made, a distribution is derived based on the estimation of five quantiles (Table 8.2). A further feature of the quantitative methodology is that different scenarios can be constructed in which the consequences of a combination of risk-reducing measures can be assessed compared with the phytosanitary measures currently in place. These may take the form of removing the current measures, increasing them, or supplementing them with additional measures (Table 8.3).

The responsibilities of EFSA in pest risk assessment are limited to the impacts on crop yields and quality, and environmental impacts. The broader socio-economic and cultural issues that are relevant for the management of risk do not fall within the domain of EFSA, but remain with the European Commission. Within its remit, the impacts on crops, on ecosystem services and on biodiversity depend on factors such as the temporal and spatial occurrence of the pathogen and its hosts in the occupied spatial units, and where available its abundance, as represented in maps at different

Table 8.2 Estimation based on quantiles and Monte-Carlo simulation

Data for parameters defined, where available, with expert knowledge elicitation
Output for each sub-step represented as a probability distribution
Input for next sub-step, with scale change where necessary
Risk-reducing options assessed through effects on parameters
Sensitivity of output to individual parameters assessed
Scenarios defined

Table 8.3 Risk-reducing options

Scenarios are based on risk reducing options to be considered
Baseline scenario, current phytosanitary regulations (A_0)
Removal of all phytosanitary requirements (A_1)
Specification of new phytosanitary requirements (A_2)
A_3 ….. A_n, other scenarios

scales, or administrative regions, such as NUTS2[1] (Prospero and Cleary 2017; Sofaer et al. 2018). Impacts on crops are assessed based on expected yield losses under current production and crop protection practices, but realising that these practices might provide extra protection against other pathogens. It is important to recognise and state the current quality criteria and thresholds to assess quality losses. These may change over time and be affected by pathogen introduction. Assessment of the relative impact on the crop is based on changes in the defined production unit, for example the individual plant or cropping area. Assessment of the impact on the environment considers two main components: the level of provision of ecosystem services and components of biodiversity (Gilioli et al. 2014, 2017a; Valenta et al. 2017). Estimation of impact on the environment considers only the percentage decreases in these two components.

For these reasons, the most challenging area in the new methodology is to assess quantitatively the impact, more generally consequences, of the introduction and spread of a quarantine plant pathogen. In many cases, in addition to crop losses, there will be cropping area, and environmental impacts to be considered in the

[1] NUTS – NOMENCLATURE OF TERRITORIAL UNITS FOR STATISTICS (https://ec.europa.eu/eurostat/web/nuts/background)

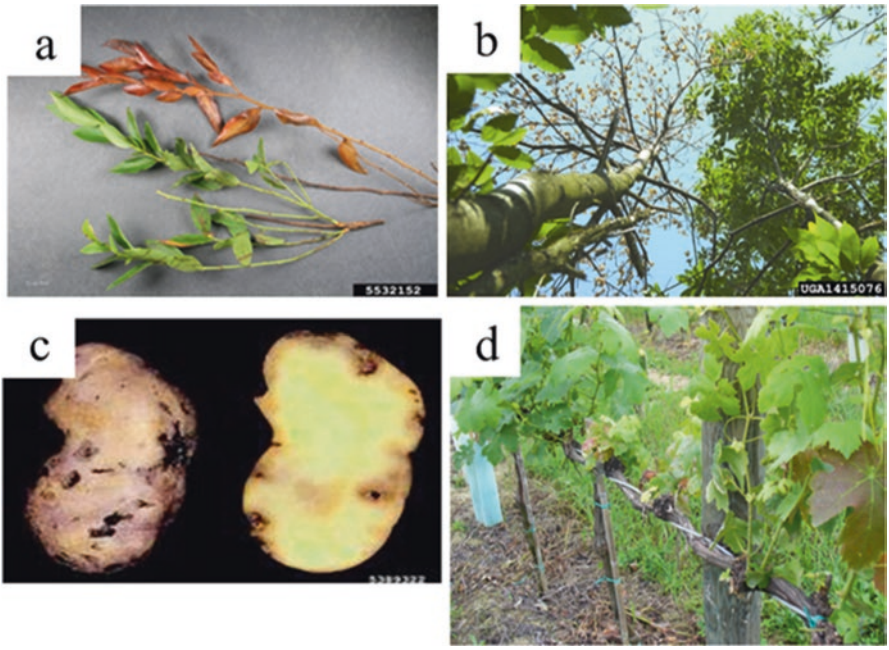

Fig. 8.2 Symptoms and damage caused by: (**a**) *Diaporthe vaccinii*, phomopsis canker and twig blight on lowbush blueberry *Vaccinium angustifolium*. (source B. Watt, University of Maine, https://www.invasive.org/browse/detail.cfm?imgnum=5532152); (**b**) *Cryphonectria parasitica*, chestnut blight in Slovakia (source Bugwood.org); (**c**) *Ditylenchus destructor*, damage on potato tuber; (**d**) Flavescence dorée phytoplasma, early season symptoms (stunted sprouting) on cv. Barbera (source IPSP-CNR Torino)

assessment (van Bruggen et al. 2018). The assessment of these impacts will be illustrated for the following examples where EFSA's quantitative methodology has been applied: *Diaporthe vaccinii* (EFSA PLH Panel et al. 2017a, *Cryphonectria parasitica* (EFSA PLH Panel et al. 2016a), *Ditylenchus destructor* (EFSA PLH Panel et al. 2016b), and the 'Flavescence doree' phytoplasma (EFSA PLH Panel et al. 2016c) (Fig. 8.2). These examples cover a range of plant pathogen taxa, host types, and importance attached to the dimensions of impact considered. In this Chapter we present only the assessment of impact for these four illustrative examples. Other examples of quantitative risk assessment of quarantine plant pathogens are published in the EFSA Journal[2] (EFSA PLH Panel et al. 2016d, 2017b and 2017c).

[2]The *EFSA Journal* is an open access, online scientific journal that publishes the scientific outputs of the European Food Safety Authority (https://efsa.onlinelibrary.wiley.com/)

Quantitative Assessment of Impact

In the quantitative pest risk assessment (EFSA Plant Health Panel et al. 2018), two types of impact are assessed using the new methodology: on the plant production system and on the environment. Each of these presents distinctive challenges for assessment depending on pathogen status in the EU (whether not known to be present; or present, but under official control) and the time horizon to be considered. The quantitative assessment is made by expert knowledge elicitation (EFSA 2014) based on available data and other information, and is expressed in the form of a probability distribution representing the uncertainties intrinsic to the assessment (Gilioli et al. 2017b). A tiered approach can be taken to ensure the levels of detail, complexity and effort of the assessment are in line with the time and resources available and the needs of the decision makers, the 'risk managers'. In the first tier approach, a direct assessment of impact is made using a "weight of evidence" approach, without resorting to modelling. This approach is currently being applied in an exercise in which the socio-economic impacts of quarantine plant pests in the EU are assessed with the aim of prioritizing EU phytosanitary measures. To support the socio-economic impact analysis, important variables such as yield loss, quality loss, disease spread rate, and time to disease first detection are estimated. A quantitative assessment of these agronomic and epidemiological variables will then provide input to the socio-economic models being developed.

In the second tier approach, the probability distribution of the risk of a plant pest is obtained by a probabilistic modelling of all the sub-steps from pest introduction to the consequences that follow. In this Chapter we describe and discuss the tier 2 approach, and illustrate its use with respect to the four examples above.

The following estimations are made in the quantitative assessment (EFSA Plant Health Panel et al. 2018):

1. Abundance of the pathogen in the spatial units occupied by the pathogen under different scenarios, reflecting current and alternative risk-reducing practices;
2. Changes in crop production outputs (crop quantity) in the spatial units occupied by the pathogen under the different scenarios, repeated as appropriate for each relevant crop use or crop habitat;
3. Changes in crop quality outputs in the spatial units occupied by the pathogen under the different scenarios, repeated as appropriate for each relevant crop use or crop habitat;
4. Relative changes in ecosystem services provision levels (provisioning, regulating and supporting services) in the spatial units occupied by the pathogen under the different scenarios (the level of ecosystem services provision with the pathogen absent in the spatial units potentially occupied under the different scenarios can be set to 1);
5. Relative changes in biodiversity (e.g. percentage reduction in species richness) in the spatial units occupied by the pathogen under the different scenarios (the level of biodiversity with the pathogen absent in the spatial units potentially occupied under the different scenarios can be set to 1);

6. Spatial units occupied by the pathogen where additional risk-reduction measures under the different scenarios are required;
7. Furthermore, there can be a stratification of the assessed variable. For instance, the distribution of the impact can be elicited for different zones.

In the second tier approach, an absolute, rather than relative estimate of the impact is made. The estimation of the absolute impact is made by considering the occupied spatial units, partitioning these spatial units according to crop use and habitat, and the estimated relative impact on yield, quality ecosystem services provision and biodiversity components. Impact is derived by considering available data and the output computed by means of models. For example, impact can be elicited by expert knowledge in relation to severity of a disease, where the severity itself is calculated by means of a model.

How impact is assessed depends on the output from the previous step of the assessment. If, for example, disease spread is modelled at the level of individual plants, the impact calculation would be based on the yield loss per plant combined with the plant density. This is relatively simple and was done in the assessment for *Diaporthe vaccinii* (EFSA Plant Health Panel et al. 2017a) and *Ditylenchus destructor* (EFSA Plant Health Panel et al. 2016b), two of the examples summarised below. If, by contrast, spread is modelled in terms of the change in spatial units occupied, e.g. the European system of "NUTS regions", then either (or both) expert elicitation and modelling would be needed to assess impact, taking into account the spatial heterogeneity in the occurrence of the pathogen and its hosts in the defined spatial units. Estimation of impact would then need to be integrated over the time horizon considered, particularly relevant for perennial crops.

Where spread is modelled based on occupied spatial units, assessment should be made of pathogen density within these areas. The issue of density is multi-scale and would concern, for example, the proportion of affected fields within a given NUTS area. It would also concern the proportion of diseased plants in affected fields (disease incidence); and finally, disease severity on the infected plants. Aggregation of diseased plants in foci, rather than in uniform or random patterns may occur. Modelling these multi-scale processes is demanding; however, in estimating the uncertainty distributions, these multi-scale issues, including spatial heterogeneity, can be taken into account and are amenable to expert judgement. One way to account for heterogeneity is to distinguish areas where the pest has established and where the host is present, such that there is potential for impact. An assumption can then be made on the density of the pest in these areas so that the impact can be calculated. A more informative way of accounting for heterogeneity is to present indicators for pathogen and host occurrence on a gridded map of the risk assessment area.

At the highest level of resolution, where experimental data are available, models for crop impact are often dose-effect relationships, where the "dose" is the incidence or severity of the pathogen, and the "effect" is the plant response in terms of yield or quality. Again interpretation and use of these relationships in estimating impact would depend on the experimental units, e.g. whether individual plants or plot/field data. For environmental impact assessment, an estimate would be based

on ecosystem functioning and ultimately the change in the level of ecosystem provisioning services (Gilioli et al. 2014, 2017a; van Bruggen et al. 2018).

We now describe the application of the quantitative approach for four contrasting examples. For the purposes of this Chapter, and to assist the general readership, the quantitative outcomes of assessment of impact are in some cases translated into qualitative expressions.

Diaporthe vaccinii

Symptoms of diseases caused by *Diaporthe vaccinii* (imperfect stage: *Phomopsis vaccinii*) on cranberries and blueberries are shown in Fig. 8.2a. *D. vaccinii* is an ascomycete that rarely produces ascospores, so that long-distance wind dispersal is rare. The imperfect form *P. vaccinii* produces pycnidiospores, which are dispersed by rain splash over short distances. Thus, movement of infected planting material is the most important means of dispersal. The pathogen is endemic in North America and occurs rarely in north-eastern Europe. Most *Vaccinium* species are susceptible.

The losses from *D. vaccinii* in northeastern and central USA over the period 1933–1983 were summarised by EPPO (1997a). Production losses of cranberries ranged from 18 to 35% and further storage losses occurred. Epidemics in blueberry fields led to twig blight causing yield losses ranging from 25 to 37% yield loss in south-eastern USA. *D. vaccinii* was associated with about 15% defective blueberry fruit in New York supermarkets. In spray trials on blueberry in North Carolina (2000–2004) reported in online Plant Disease Management Reports of the American Phytopathological Society, there were 5% blighted blueberry twigs with the best fungicide treatment and 28% in untreated plots; at harvest, there was 9% fruit rot with fungicide treatment and 12.4% without (EFSA PLH Panel et al. 2017c; van Bruggen et al. 2018). Relation between per cent of twigs blighted and yield loss per blueberry bush, derived from eight fungicide spray trials in North Carolina from 2000 to 2003, were reported in Plant Disease Management Reports of the American Phytopathological Society by W. O. Cline, B. K. Bloodworth (NC State Univ.) and C. W. Meister (Univ. Florida).. As *D. vaccinii* can have a severe impact on blueberry in parts of the USA (Weingartner and Klos 1975), it could present a problem if introduced in southern regions of Europe (Narouei-Khandan et al. 2017). In similar reports on cranberry experiments in Wisconsin and Massachusetts, there was a 76% yield loss from all fungal diseases (EFSA PLH Panel et al. 2017c; van Bruggen et al. 2018); however, losses directly attributable to *D. vaccinii* were not assessed. Storage rot incidence of *D. vaccinii* varied from 0% to 15% (Olatinwo et al. 2004). In Canada, *D. vaccinii* was prevalent with 13% fruit infection at harvest (Sabaratnam et al. 2016). In Latvia, *D. vaccinii* was frequently isolated from decaying cranberry fruit (Vilka et al. 2009). Cranberry fruits are highly susceptible to *D. vaccinii* and there is the potential for impact if introduced into northern regions of Europe (Michalecka et al. 2017).

Of the native *Vaccinium* species in North America, *V. angustifolium* and *V. arboretum* are relatively resistant to *D. vaccinii* (Polashock and Kramer 2006), with disease on these species generally low in wild habitats. The incidence of *D. vaccinii* isolated from rotten fruits in native cranberry stands could be as high as 23% (Stiles and Oudemans 1999). *D. vaccinii* has been reported only occasionally in northern Europe (EFSA PLH Panel 2014b; Lombard et al. 2014; EPPO 2015; Vilka and Volkova 2015; EFSA PLH Panel et al. 2017a; Michalecka et al. 2017). As a consequence, native European *Vaccinium* species have rarely been exposed, so a worst-case scenario would be *D. vaccinii* establishing in susceptible wild stands (Narouei-Khandan et al. 2017). However, the ability of *Vaccinium* plants to regrow after twig blight and dieback make it unlikely that *D. vaccinii* infection alone would have impact in northern Europe as stem symptoms induced by *Fusarium putrefaciens* are common in wild *Vaccinium* stands (Strømeng and Stensvand 2011).

Assessment of Impact

Full details of the quantitative risk assessment (for entry, establishment, spread and impact) are given in EFSA PLH Panel et al. (2017a); here, we summarise only the assessment of impact. As the import of *Vaccinium* plants into the EU and the climate suitability for *D. vaccinii* varies considerably across the EU, the potential impact was assessed separately for four regions, the north-west, the north-east, the south-west and the south-east (EFSA Plant Health Panel et al. 2017a). Pathogen spread within regions was modelled as a combination of the movement of infected plants and by splash dispersal within fields. Impacts were assessed as loss of plants and of berries in the four regions. Three situations were assessed: nursery production of propagated or ornamental plants, commercial berry production, and natural stands. Three scenarios were considered: A_0, the current regulatory status; A_1, delisting as a quarantine organism; A_2, more stringent requirements, such as imported plants originating directly from tissue culture. The relevant time horizon was considered to be 5 years. In the first instance the impacts under A_0 were assessed.

Vaccinium Production in Nurseries

With the current legislation (scenario A_0), detection of a *D. vaccinii* infection leads to eradication and the loss of the complete production lot. Affected nurseries would be closed for a period of three or more months, the potential incubation period (van Bruggen et al. 2018). Under scenario A0, the number of outbreaks in nurseries would be expected to be higher in the north-east and south-west EU (some 20–130 plants infected per year in 5 years) compared to the north-west and south east (0–17 infected plants). This difference between regions reflects the difference in estimated trade flow of small plants to these areas, favourable climatic conditions (in the north-east), and presence of significant berry production.

Commercial Berry Production

Plants in production fields may be infected by other *Phomopsis* species present in Europe that induce similar symptoms to *D. vaccinii*. It was assumed that some *D. vaccinii*-infected plants would be missed by the farmer, removal of these plants would not take place, and an outbreak with rapid spread might result, especially with overhead irrigation or mechanical harvesting. It was also assumed that *D. vaccinii* would be partly controlled with standard fungicide applications, but there would be the need for additional fungicide applications. Under scenario A_0, the highest estimated number of infected plants after 5 years in production areas of the north-eastern region would range from 70 to 440 infected plants. Poland is an important blueberry producer with 2500–5000 ha of blueberry production, between 7.5 and 15 million plants. If all introductions took place in Poland, the estimated number of infected plants would be extremely low. Hence, the expected impact of *D. vaccinii* in commercial berry production under scenario A_0 would be low, although locally, additional fungicide treatments may be needed and some loss in production would be endured due to replacement of infected plants.

Natural Areas

Vaccinium species are widespread and a valued component of ecosystems in natural and semi-natural areas in the EU. *Vaccinium* species provide provisioning services (consumption of wild berries), regulating services (pollination and erosion protection), supporting services (nutrients and water cycling), and cultural services (recreation and tourism). There is limited information on the host suitability of native *Vaccinium* species in the EU territory. Eradication of *D. vaccinii* in the natural environment would be difficult to achieve without removal of all *Vaccinium* plants in an outbreak area. However, infected plants can recover to some extent from the stolons (cranberries) or from roots and lower stems (blueberries) provided disease severity is limited (van Bruggen et al. 2018). Under scenario A_0, the estimated number of infected plants in the natural environment would be highest in the north-eastern EU (100–400,000 infected plants in 5 years) compared to other regions (0–10,000 infected plants).

Uncertainties Affecting the Assessment of Impact

The undetected presence of *D. vaccinii* in nurseries may lead to its spread, through plant movements, to retail garden centres, production fields and other nurseries. Impact of *D. vaccinii* on berry production in organic blueberry and cranberry farms, where the farmer relies mainly on removal and replacement of infected plants, may carry the potential for further spread and consequently multi-year yields. The host suitability of wild European *Vaccinium* species and the level of mortality induced by *D. vaccinii* is unknown.

Conclusions on Impact for the Different Scenarios

The predicted impact of *D. vaccinii* in the EU after a 5-year period for the three scenarios is summarised in Fig. 8.3 for nurseries, production fields and natural areas.

Under scenario A_1, where *D. vaccinii* is no longer listed as a quarantine organism, the number of infected plants in nurseries is estimated to be 20 times higher compared to scenario A_0. The main reason is that the presence of *D. vaccinii* in third-country and EU nurseries does not necessarily lead to rejection of consignments or subsequent eradication measures. Under scenario A_2 with more stringent requirements such as imported plants exclusively originating from tissue culture, the number of infected plants in nurseries would be effectively reduced to zero.

Under scenario A_1,the number of infected plants per year in production areas is expected to increase by a factor of 80–100 compared to scenario A_0 as new plants planted in production areas may be infected with *D. vaccinii* and no obligatory eradication measures would be in place. Under scenario A_2 with more stringent requirements for plants for planting, the number of infected plants per year in production areas would be close to zero.

Under scenario A_1, the number of infected plants per year in natural areas is expected to increase by a factor of three compared to scenario A_0, due mainly to the establishment of a greater number of founder populations. This increase might negatively affect the ecosystem services *Vaccinium* provides; however, it is uncertain if infection would lead to a decrease in the density of wild *Vaccinium*. Under scenario A_2 with more stringent requirements for plants for planting, the number of infected plants in natural areas would reduce substantially.

Cryphonectria parasitica

Cryphonectria parasitica is the causal agent of chestnut blight in North America and Europe, a destructive disease that kills trees through bark cankers (EFSA PLH Panel 2014a; Rigling and Prospero 2018). Symptoms of disease caused by this fungus are shown in Fig. 8.2b. Impact largely depends on the presence of hypovirulence arising from virus infection in the fungal population (Anagnostakis and Jaynes 1973). With no hypovirulence, the impact can be considerable; whereas with hypovirulence it is contained (Griffin 1986). In Europe the frequency of hypovirulence can vary: in Italy, it occurs in about 90% of chestnut stands; in France and Portugal, it is considerably lower (Colinas and Uscuplic 1999; Bragança et al. 2007). New virulent strains that lack hypovirulence, either introduced from outside the EU territory or formed within the EU population by mutation or sexual recombination (Ježić et al. 2012), could spread and have considerable impact. Any increase in vegetative compatibility diversity of *C. parasitica* in the EU would limit virus transmission and hence the establishment and further spread of hypovirulence (Turchetti and Maresi 2008), as has been the experience in the USA (Milgroom and Cortesi 2004). Hypovirulence would be a promising approach to reducing the impact of

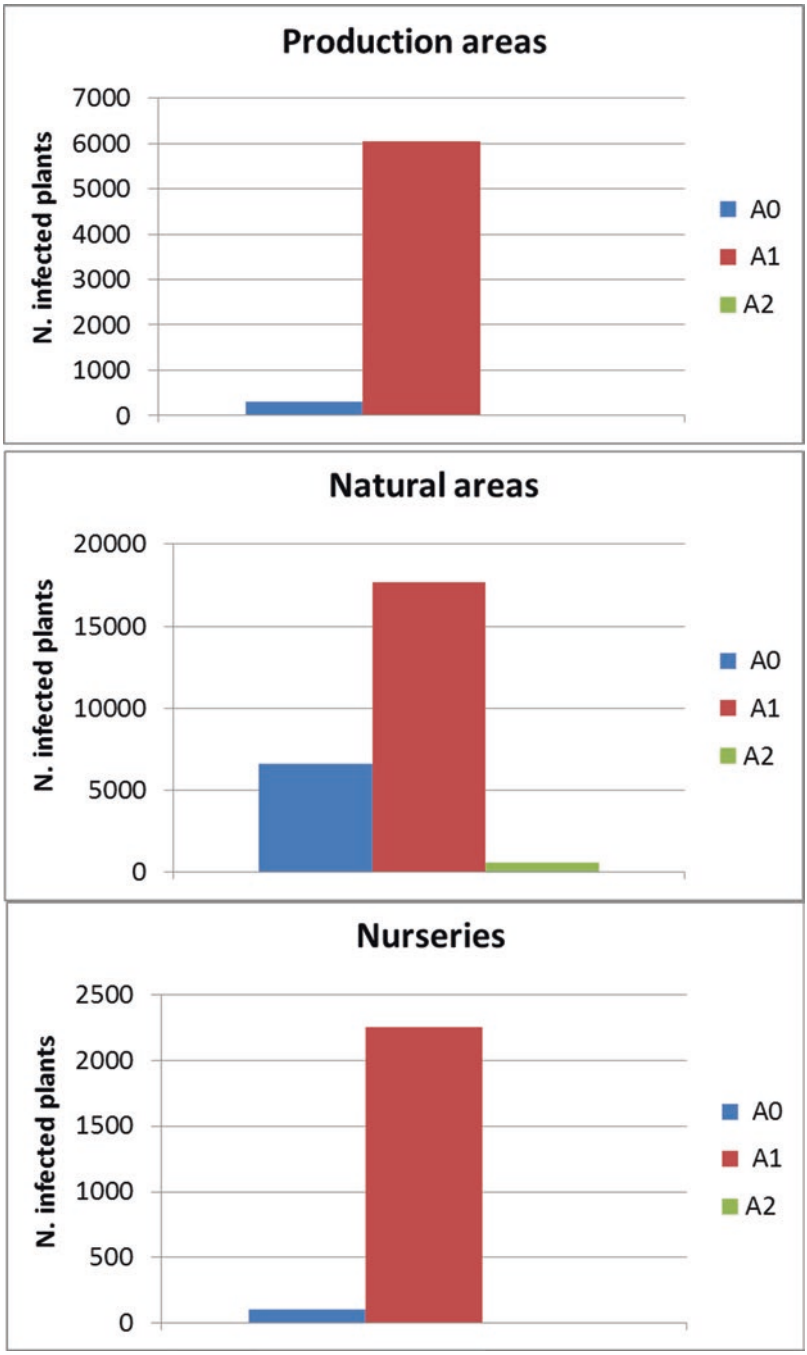

Fig. 8.3 Number of infected plants per year, after a 5-year period, in the production areas (blueberry and cranberry cultivations), natural areas and plant nurseries for scenarios A_0, A_1, A_2 for *Diaporthe vaccinii*

C. parasitica as hypovirulent strains do not generally kill infected trees; furthermore, hypovirulence would be able to establish and spread and eventually become predominant in the population (Heiniger and Rigling 1994; Turchetti and Maresi 2008). It may be possible to introduce hypovirulence by artificial inoculation (Heiniger and Rigling 1994, 2009; Prospero and Rigling 2016).

Assessment of Impact

Full details of the quantitative risk assessment for each step of the approach (entry, establishment, spread and impact) are given in EFSA PLH Panel et al. (2016a); here, we summarise only the assessment of impact. Assessment of impact was made for virulent strains only, because hypovirulent strains do not cause relevant damage to plants. Two situations were analysed: the first, based on the situation in the EU before the appearance of hypovirulence, concerns the damage recorded in the 1960s; the second, based on the current hypovirulence situation in the EU, recognizing that this is not uniform (EFSA PLH Panel et al. 2016a), in which a low level of damage occurs. The risk of impacts due to *C. parasitica* were assessed under three scenarios A_0 (the current regulatory status), A_1 (de-listing) and A_2 (additional risk-reducing options based on Protected Zone status in the EU) and focused on the virulent strains of the pathogen, able to overcome hypovirulence (EFSA PLH Panel et al. 2016a). The time horizon of the assessment was 10 years. The main parameters considered in assessing impact were: the proportion of newly introduced strains of *C. parasitica* that are virulent; the proportion of the area with presence of *Castanea sativa* the main host *Castanea sativa* affected by new virulent strains; and the impact on ecosystem services. The proportion of new strains of *C. parasitica* that are virulent was estimated by expert judgment, based on information on the historical number of pathogen entries between the first introduction into the EU and the present time, and assuming that the proportion does not change over the 10-year period.

The impact was assessed by considering the proportion of the area with the main host *C. sativa* present and affected by new virulent strains. The proportion was derived from a simple model based on a logistic growth which described the disease dispersal dynamics. The impact on services, expressed as a proportionate reduction, was assessed for provisioning, regulating, supporting and cultural ecosystem services. The assessment was made for the proportion of the area with the main host present where new virulent strains were expected to have spread by the end of the 10-year assessment period.

The estimated proportion of newly introduced strains of *C. parasitica* that are virulent was assumed to be the same for all scenarios and to range from 0.1% to 0.5%, with a median value of 0.3%. The median area with presence of *C. sativa* and affected by new virulent strains of *C. parasitica* under scenario A_1 (about 58%) was estimated as about twice that for scenario A_0 (about 31%); the estimate for scenario A_2 (about 9%) was about three times lower than for scenario A_0. The values obtained in scenario A_1 indicate that in case of introduction and spread of new virulent strains

the currently effective hypovirulence present in native populations would be compromised, resulting in a large impact.

The estimated relative impact on ecosystem services as a result of introduction and spread of new virulent strains was found to be consistent for the different ecosystem services for each scenario. Nonetheless, in absolute terms, the estimated impacts on ecosystem services were higher for scenario A_1 compared to scenarios A_0 and A_2, as the proportion of the area with the main host and new virulent strains present was larger. Although damage caused by newly introduced virulent strains of *C. parasitica* would affect the ecosystem services provided by *C. sativa*, some services could be recovered through recolonization by new tree species (Boyd et al. 2013). However, the cultural importance attached to ancient chestnut trees cannot be replaced by new plantings of other species (Buonincontri et al. 2015). *C. sativa* is iconic, widely distributed and locally abundant in forest ecosystems of the EU, particularly in Mediterranean countries (Mellano et al. 2012). The loss of this tree species would lead to a reduction in ecosystem services; and potentially to a loss in the biodiversity associated with chestnut, although there is currently insufficient knowledge to quantify such a reduction. On the other hand it is also possible that by replacing homogeneous chestnut woodland with more diverse plantings of tree species, the biodiversity of some taxa could increase.

Uncertainties Affecting the Assessment of Impact

The main factors contributing to the overall uncertainty in the assessment were the initial frequency of new virulent strains, the estimated dispersal rate of *C. parasitica*, and the level of effectiveness of risk-reducing measures. In all cases, the most important factor was the initial abundance of virulent strains. A factor that was not considered explicitly in the assessment, but which may well be important in determining the impacts of new virulent strains of *C. parasitica*, is the genetic diversity of the host *C. sativa*, which shows strong geographical structure in Europe (Mattioni et al. 2013).

Conclusions on Impact for the Different Scenarios

The impact of the introduction of new virulent strains of *C. parasitica* into the EU is summarised in Fig. 8.4 for the three scenarios assessed. The width of the estimated distributions related to the proportion of the area with the presence of the main host and where new virulent strains of *C. parasitica* will be present after 10 years is wider under scenario A_1 compared to scenarios A_0 and A_2, which implies that the uncertainty on impacts is lower for the latter two scenarios.

Although *C. parasitica* is already widely distributed in the EU, there is the potential for greater impact due to new introductions of strains from East Asia, the native range of the fungus, and North America, where the fungus has caused the near extinction of the native *Castanea dentata*. This conclusion is also valid for other

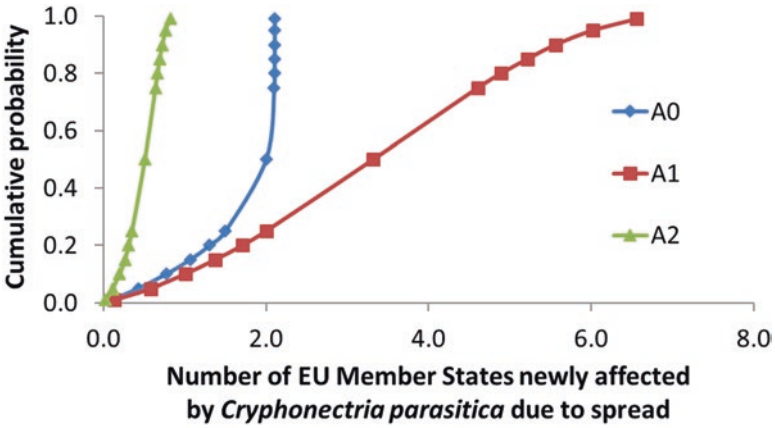

Fig. 8.4 Cumulative probability of EU Member States affected by *Cryphonectria parasitica* after 10 years of spread in three scenarios, A_0, A_1, A_2

pathosystems such as ash dieback due to *Hymenoscyphus fraxineus* (Landolt et al. 2016; McMullan et al. 2018). As shown by this risk assessment, removing the current phytosanitary status of *C. parasitica* would increase the diversity and virulence of pathogen populations, leading in turn to much higher impacts on chestnut in the EU territories.

Ditylenchus destructor

The damage to potato tubers caused by this nematode is shown in Fig. 8.2c. The foliar symptoms caused by *D. destructor* are often missed (Sturhan and Brzeski 1991) when sampling in infested fields, leading to some uncertainty as to the colonization of affected plants (MacGuidwin et al. 1992), especially as environmental conditions may affect the expression of symptoms. Damage has rarely been reported from crops other than those vegetatively propagated, notably for potato (Sturhan and Brzeski 1991) which *D. destructor* affects adversely in both yield and quality (EFSA PLH Panel 2014c).

Potato is one of the most important food crops globally (Hijmans 2001). The potato production area in the EU was 1.64 million ha in 2014 with a potato tuber yield of almost 46 million tonnes (EUROSTAT online). The seed potato production area in the EU in 2014 was about 109 thousand ha, some 7% of the potato production area with a yield estimated at 25 tonnes/ha (ESCAA online). Although *D. destructor* was reported to be an important pest of potato in temperate regions (Sturhan and Brzeski 1991), reducing yield and quality, losses were mainly observed before 1970 in Europe. The reasons for this may include seed certification (Sturhan and Brzeski 1991) and the inspection requirements for other regulated pests such as

Clavibacter michiganensis subsp. *sepedonicus*. The distribution of the pest is not known in detail but the majority of EU Member States have reported the presence of *D. destructor*.

According to Mwaura et al. (2014, 2015), the main impact on yield is through reduction in the weight of tubers, depending on cultivar response to infection and nematode population density; at low densities quantifiable yield loss does not occur. Yield losses are likely to differ depending on whether the source of infection is infested seed tubers or nematodes present in the soil. Some risk-reducing measures are available to suppress populations of *D. destructor* in the soil. Fumigation is most effective in moist soils that are well drained and contain little clay or organic matter (Whitehead 1998), but today, other than in certain circumstances, is not practised in the EU.

Assessment of Impact on Potatoes

Full details of the quantitative risk assessment for each step of the approach (entry, establishment, spread and impact) are given in EFSA PLH Panel et al. (2016b); here, we summarise only the assessment of impacts on potato. Scenarios assessed were A_0 (current regulation), A_2 (pest-free place of seed potato production) and A_6 (use of soil fumigation); the numbering of scenarios as used in the EFSA report (EFSA PLH Panel et al. 2016b) was kept in this chapter. Two types of impacts of *D. destructor* on potato production (EFSA PLH Panel et al. 2016b) were assessed:

- Reduction in the quantity of potatoes produced due to the effect of nematodes on the growth of the plant. This is mainly relevant for ware potatoes, representing more than 90% of all potatoes produced in the EU.
- Reduction in the market value (quality) of potatoes produced in an infested field due to the presence of nematodes in the product, particularly important for seed potatoes.

For each type of impact, two situations were assessed.

Yield Loss Arising from Nematode Infestation of the Soil

This occurs when healthy seed potato tubers are planted in infested fields leading to infection by *D. destructor*. The yield loss due to infection of potato plants from soil was calculated by assessing the total number of plants infected in this way across the EU. The proportionate yield loss was estimated on the basis of available literature and expert judgement.

Yield Loss Due to Infection of the Seed

This occurs from planting infested seed potato tubers irrespective of pest infestation in the field. The yield loss was calculated by multiplying the total number of infested tubers planted each year across the EU by the expected yield per plant and a factor expressing the proportionate yield loss, estimated using expert judgement.

Uncertainties in the Assessment of Impact for Potatoes

Sensitivity analysis showed that 87% of the overall uncertainty in yield loss of potatoes would be due to uncertainty in the proportion of infested potatoes in infested fields. The impact pathway from the soil would be more important than the impact pathway from the seed. Impact in terms of quality losses was estimated to be negligible; but with some uncertainty because there are no reporting requirements when infestations are detected before seed certification.

Overall Conclusion on Impact

The overall impact of *D. destructor* on potato production is summarised in Fig. 8.5 for the three scenarios assessed.

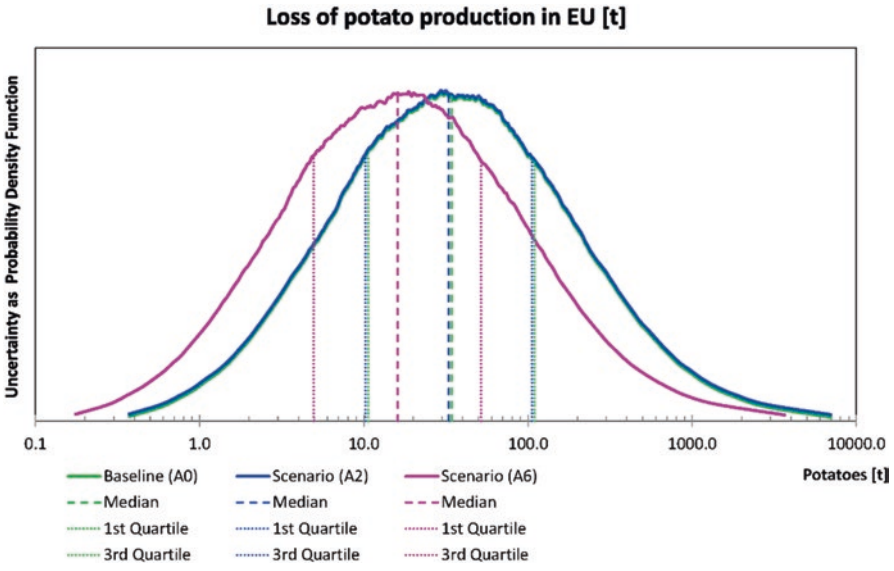

Fig. 8.5 Estimations of yearly losses of potato production in the EU following the introduction of *Ditylenchus destructor* under different scenarios (A_0, A_2, A_6). Lines for A_0 and A_2 are not distinguishable on the scale shown. From EFSA PLH Panel et al. (2016b)

Ware Potato Production

The impact on ware potato production under scenario A_0 was estimated as a median loss of 33 tonnes per year across the whole EU. This estimate is approximately that of the average EU yield of a single potato field of 1 ha (32 tonnes/ha). The total potato production area in the EU is 1.7 million ha. The uncertainty interval from the 25 to 75 percentiles was 10–105 tonnes, and at the worst case of 99 percentile 1780 tonnes, indicating very low uncertainty under current regulations. Production of seed potatoes in pest-free places of production in those countries importing into the EU (scenario A_2) does not change the estimated impact. Soil fumigation in the field (scenario A_6) results in a 48% reduction in median impact, but whether this reduction is relevant is questionable given the minimal impact under the baseline scenario A_0.

Market Value of Seed Potatoes

Damage to seed potatoes caused by the nematode would result in downgrading as there is a zero tolerance, but it is not known how often this occurs in the EU. Sampling and testing procedures for *D. destructor* are not specified in the EU legislation, hence detection of the nematode would mostly arise from the finding of rotten tubers. Asymptomatic infestation would not normally be detected. The impact in terms of downgrading of seed-potato lots would be of minimal importance under current conditions (A_0) and would not change with delisting because of the current EU legislation on seed-potato certification. With the intensified measures considered (scenarios A_2 and A_6) a reduction in the impact in seed potato production is conceivable but would be of minimal importance.

The impact of the nematode on the quantity and quality of the yield was assessed as negligible for ware potato and highly restricted for seed potato. The nematode is a pest of agricultural and horticultural crops and is not known to impact ecosystem services, biodiversity or the environment. Impact under the current legislation (A_0) is considered low and it is not likely that removal of current nematode-specific regulations would increase impact.

Flavescence dorée Phytoplasma

Flavescence dorée phytoplasma (FDp) is a phytoplasma with a narrow host range. Symptoms of Fdp in grapevine caused by the phytoplasma are shown in Fig. 8.2. Before its emergence as a pest of grapevine and wild *Vitis* spp. in Europe, it was known to infect alder (Alnus spp.) and Clematis spp. but without significant damage (Filippin et al. 2007; Malembic et al. 2007). The phytoplasma is transmitted from plant to plant by leafhoppers or planthoppers which occasionally infect *Vitis* spp.

without causing an outbreak (Filippin et al. 2009; Mehle et al. 2010; Gaffuri et al. 2011; Trivellone et al. 2016). The invasive leafhopper *Scaphoideus titanus* was introduced from North America and first observed in France in 1958 (Bonfils and Schvester 1960). This species is restricted to cultivated and wild *Vitis* spp. (Lessio et al. 2007). Wild *Vitis* spp. and hybrids are mostly susceptible to FDp (Moutous 1977; Eveillard et al. 2016), but remain symptomless (Caudwell et al. 1994). *S. titanus* has so far spread to 11 EU Member States (Tothova et al. 2015). *S. titanus* is responsible for the epidemic spread of FDp in vineyards once the phytoplasma has been transferred from alternative reservoir plants by other vectors (Filippin et al. 2009; Mehle et al. 2010; Gaffuri et al. 2011; Trivellone et al. 2016).

Removal of infected plants (roguing) is a major component of disease control strategies (EFSA PLH Panel 2014d), reducing the risk of local disease spread (Bressan et al. 2006). Abandoned vineyards are potential sources of infective *S. titanus*, first observed in France in 1958 (Bonfils and Schvester 1960). This species is restricted to cultivated and wild *Vitis* spp. (Lessio et al. 2007). Wild *Vitis* spp. and hybrids are mostly susceptible to FDp (Moutous 1977; Eveillard et al. 2016), but remain symptomless (Caudwell et al. 1994). *S. titanus* has so far spread to 11 EU Member States (Tothova et al. 2015). *S. titanus* is responsible for the epidemic spread of FDp in vineyards once the phytoplasma has been transferred from alternative reservoir plants by other vectors (Filippin et al. 2009; Mehle et al. 2010; Gaffuri et al. 2011; Trivellone et al. 2016). which can then re-colonize the cultivated vineyards (Pavan et al. 2012a; Lessio et al. 2014). Wild species may reach up to 6 m in height, providing a dense and shaded habitat for *S. titanus* (Cravedi et al. 1993). In the absence of control measures, *S. titanus* can achieve high population sizes leading to epidemics of FDp (EPPO 1997b; Pueyo et al. 2008). Several chemical families are used against *S. titanus* nymphs and adults with varying efficacy (Chuche and Thiéry 2014; Gusberti et al. 2008; Sivčev et al. 2010). Insecticide applications against *S. titanus* are compulsory in EU Member States where both the vector and FDp are present (EFSA PLH Panel 2014d), although their frequency varies between countries (Belli et al. 2010). Hot-water treatment of dormant canes is highly effective in eliminating FDp (Caudwell et al. 1997; Bianco et al. 2000; Mannini and Marzachì 2007) and *S. titanus* eggs on propagation material (Linder et al. 2010). The original introduction of *S. titanus* in Europe is thought to have occurred via imported grapevine canes carrying eggs (Bertin et al. 2007) and trade of planting material contributes to the dissemination of FDp and *S. titanus* (Weintraub and Beanland 2006).

Although all *V. vinifera* varieties are susceptible, they differ in symptom expression and severity. Some rootstocks bred from North American *Vitis* species may remain symptomless (Schvester et al. 1967; Caudwell et al. 1994; Eveillard et al. 2016) and may provide potential sources of tance, including vector resistance (Eveillard et al. 2016). Susceptible varieties may exhibit an irregular sprouting (Morone et al. 2001; Roggia et al. 2014) with symptoms on leaves, death of inflorescences and berries, following later in the season (Caudwell 1990). Death of the affected grapevines (Credi 1989; Morone et al. 2007; Pavan et al. 2012b) can follow,

although in some varieties a remission of symptoms has been observed. The lowest impact would be seen on a plant infected late, although damage may accrue in following years. The median of infected grapevine in the year of infection has been estimated at 60% (Credi 1989).

Assessment of Impact

Full details of the quantitative risk assessment for each step of the approach (entry, establishment, spread and impact) are given in EFSA PLH Panel et al. (2016c); here, we summarise only the assessment of impact. A quantitative assessment of the impact on wine and table grape production was made under scenario A_0, the current regulatory status. The assessment took into account five parameters (EFSA PLH Panel et al. 2016c) and covered a 10-year time horizon:

1. the number of infested NUTS 2 regions;
2. the average area (ha) under grapevine production in NUTS 2 regions for table or wine-producing grapes for those regions with grape production;
3. the average incidence (% infected plants) of Flavescence dorée phytoplasma in table and wine grape production in infected NUTS 2 regions;
4. the average grape production (tonnes) in NUTS 2 regions of table and wine grapes, for those regions with grape production;
5. A conversion factor providing an estimation of the loss of production of individual grapevines as a consequence of infection.

In the absence of the vector *S. titanus*, epidemic development would not be expected in grapevine. Historically, introduction of *S. titanus* has always preceded the establishment of the disease by a few years, so that vector presence would be expected as disease spreads. Estimates of the loss of production as a consequence of infection were made in line with the current legislation (A_0) in which the removal of infected plants upon discovery is mandatory. Where new grapevines are replanted immediately, the new plants will not enter production for 2–3 years. In the worst case, production would be completely lost for 3 years and limited during the fourth year. Lastly, the control measures in place involve the complete removal of plots with more than 20% infections, resulting in additional losses in production from healthy plants. These factors were integrated in the multi-year estimation of the production losses in infested NUTS 2 regions.

The scenario in which the current phytosanitary regulations were removed was not assessed. Instead alternative scenarios considered (A_1 and A_2) were each concerned with the impact of additional phytosanitary measures. Scenario A_1 included a generalised hot water treatment that reduces FDp infection in grapevine propagative material. Scenario A_2 included more intense surveillance, containment and eradication efforts, these would: (i) allow rapid and more effective elimination of FDp where there is limited FDp prevalence; and (ii) limit vector-mediated spread between adjoining NUTS 2 regions. The average values used for the grapevine production area and grape production in NUTS 2 regions were taken as the same as those for A_0. The average incidence of FDp in infested NUTS 2 regions would be

affected by both A_1 and A_2 scenarios and specific probability distributions for this parameter were estimated for each scenario. of production of individual grapevines was considered to be unaffected or only marginally affected by the additional measures specified in A_1 and A_2. The component of production likely to be most affected is the proportion of plots reaching >20% infection; this would be expected to be reduced under the more stringent containment and eradication measures in A_2. For scenario A_2, the value for the upper 99% percentile was considered likely to be affected and a revised probability distribution incorporating this minor change was used.

Uncertainties in the Assessment of Impact

Scenario A_0

Sensitivity analysis showed that the parameter associated with the largest uncertainty was the average incidence of FDp in affected NUTS 2 regions. Estimation of this parameter represents a prediction over a period of 10 years, with no data available at an EU-wide integration scale. The assessment was based on expert judgement alone. The second parameter that contributed to the overall uncertainty was the average grapevine production area in infested NUTS 2 regions. These regions vary widely in vineyard coverage and do not easily allow estimation of average crop area values over the 10-year period. The number of infested NUTS 2 regions at the 10-year time horizon, the average grape production in individual regions, and the conversion factor representing yield in infected plants contributed less to the overall uncertainty. The main sources of uncertainty affecting the probability distribution of the conversion factor were:

- The multiyear nature of yield loss in a perennial crop,
- Estimation of the average loss of production in the year of infection,
- Estimation of the average time needed for replanted vines to enter their production phase,
- The impact of recovery on yield losses.

Alternative Scenarios

In the assessment of impact, some parameters were taken as identical to those used for A_0. The uncertainties affecting most parameters are the same as for A_0. The parameter that would be affected by the alternative scenarios is the incidence of FDp. As found for scenario A_0, this parameter has most effect on the overall uncertainty of impact. In addition, the alternative scenarios involve implementation of additional risk-reducing measures and both their combined effectiveness and feasibility carry uncertainty. Despite these uncertainties, implementation of the added or reinforced risk-reducing measures in A_1 and A_2 would reduce impact as compared to A_0.

Conclusions on Impact of Flavescence dorée Phytoplasma Under the Different Scenarios

The overall impact of FDp on grape production in the EU is summarised in Fig. 8.6. The assessment provides an estimate of the impact on wine and table-grape production under scenario A_0. The consolidated median loss for all types of grape production over the EU was estimated at close to 8000 tonnes of grapes; however, the 50% uncertainty interval spans a range of nearly two orders of magnitude, from about 1000 tonnes to 50,000 tonnes. These values represent a small proportion of the EU table- or wine-grape production. The upper impact estimates provided by the 90% uncertainty interval represent around 0.5–1% of the EU production.

There would be compulsory hot-water treatment for nurseries in infested NUTS areas under scenario A_1, and this reduced the probability of infection in traded grapevine plants for planting, a pathway for the spread of FDp. This measure has high feasibility because its implementation does not face important technical hurdles. The more intense eradication and containment measures under scenario A_2 would limit epidemic spread development. Increased surveillance and eradication, by targeting abandoned vineyards and the wild grapevine populations, would contribute to the overall effectiveness of A_2, although there may be environmental impacts. The feasibility of this scenario may be more limited than the additional risk-reducing measures in scenario A_1.

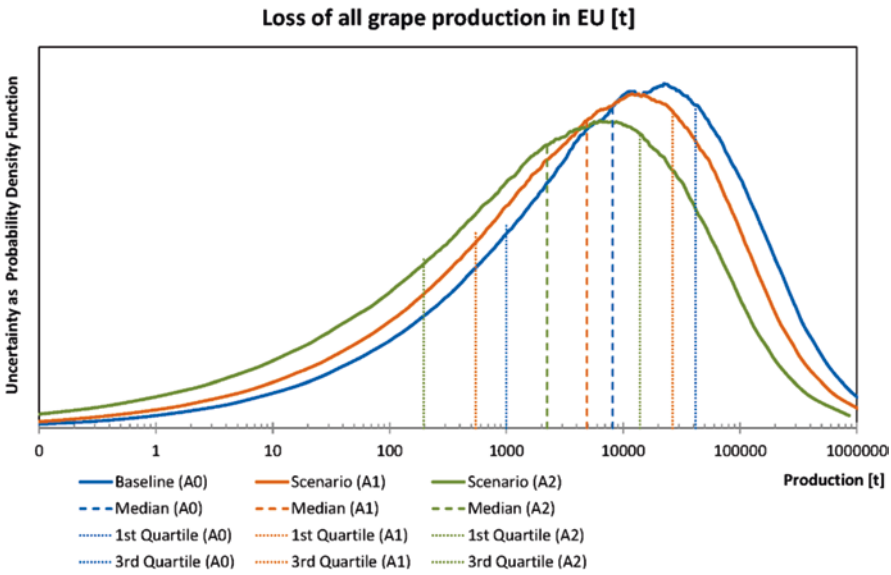

Fig. 8.6 Comparison of losses in grape production caused by Flavescence dorée phytoplasma (in tonnes, logarithmic scale) estimated for different scenarios: In blue baseline, in red scenario 1, and in green scenario 2. Shown are the density function of the uncertainty (solid lines), the median (dashed lines), and the uncertainty ranges (dotted lines). From EFSA PLH Panel et al. (2016c)

Under A_1, median impact is estimated at close to 5000 tonnes of production, about half of the estimate for A_0. The 50% uncertainty interval ranges from a few hundred tonnes to about 30,000 tonnes. Under A_2, the median impact is estimated at close to 2500 tonnes, more than a threefold reduction in the estimate for A_0. The 50% uncertainty interval ranges from about 100 tonnes to 15,000 tonnes.

Other considerations, not assessed quantitatively, included: FDp infection may sometimes have an impact on grape or wine quality but reductions in crop quality would have a minor impact compared with the direct quantitative impact on production. FDp has the potential to impact grapevine nursery production as detection of infection in a nursery would result in loss of the Plant Passport for the complete production lot. FDp is not known to cause any significant damage to alternative hosts. As a consequence, impact on ecosystem services provision levels would be limited. The risk-reducing measures in A_2 may involve the removal of wild *Vitis* spp., resulting in losses of the relatively rare ancestral undomesticated species *Vitis sylvestris*.

Comparison of the Impacts for the Four Examples

The quantitative methodology developed for the assessment of impact permits comparisons to be made across quarantine pathogens, although different time scales may be involved. For example, the estimated yield losses for three of the examples, *D. vaccinii*, *D. destructor* and Flavescence dorée phytoplasma, are shown in Fig. 8.7. The case of *C. parasitica* is different in the sense that impact was assessed

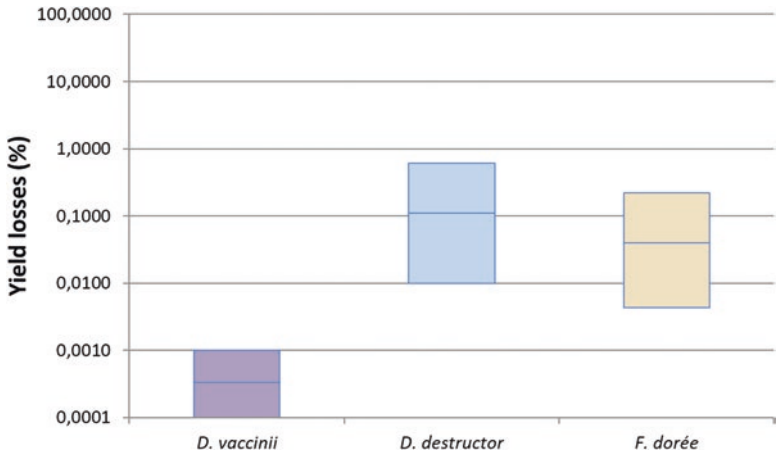

Fig. 8.7 Estimated yield losses caused by *Diaporthe vaccinii*, *Ditylenchus destructor* and Flavescence dorée phytoplasma according to scenario A_0. The estimated yield losses have been calculated on blueberry (*D. vaccinii*), potato (*D. destructor*) and grapes (Flavescence dorée) and refer to the entire EU crop production. Percentage losses are presented in the graph on a logarithmic scale, showing for each disease the median value (middle horizonal line), the 25% lower quantile (bottom line) and the 75% upper quantile (top line)

in terms of introduced virulent strains and the spatial extent of invasion over a 10-year period. A factor was used to change chestnut nuts and wood production outputs in relation to virulent strains. At the EU territory level, the estimated losses under the current regulatory regime remain relatively small, although these estimates should be interpreted in relation to the overall cropping area and production level and the concentration of production across Member States. Potato, for example, is widely grown throughout the EU territory, although seed potato is more concentrated; grapevine, for table grapes and wine production, is more restricted both across and within Member States; and *Vaccinium* cultivation (as opposed to wild stands) for berry production is even more restricted.

Overall Conclusions

The quantitative assessment of impacts of quarantine plant pathogens on crops and ecosystem services should consider many aspects, including factors affecting entry, establishment and spread, as well as the effects on host plants and ecosystems once the pathogen has established in a given locality. Impacts are temporally and spatially heterogeneous, resulting in an assessment target that, although challenging to pin down, follows logically from the preceding steps of entry, establishment and spread. Impacts evolve over the course of an invasion as the geographic range of the invasive organism expands, its density increasing and perhaps later decreasing until some kind of dynamic equilibrium is reached. Spread and impact processes are affected by responses of human managers as well as ecosystems (Pezzi et al. 2011). Ecosystems may show resilience to invasive organisms to a greater or lesser degree, resulting in varying impacts over time (Döring et al. 2015). The assessment of impacts is multi-scale, and any assessment is necessarily a simplification (Holt et al. 2017). Certainty cannot be achieved, but an assessment of uncertainty is possible, by explicitly including uncertainty as part of the quantitative approach. Thus, rather than assessing uncertainty as an add-on to qualitative assessment of risk, risk and the associate uncertainties are assessed together through the use of the estimated distributions.

The developed methodology was implemented to elicit quantitative statements on uncertainty. Scenarios based on current measures and alternatives are critical to explore possible introduction and spread pathways and their consequences (Douma et al. 2016). The quantitative methodology described here uses explicit quantification and uncertainty analysis in its assessment process for the EU territory (Gilioli et al. 2017b; EFSA PLH Panel et al. 2018). As with any new methodology, this quantitative methodology will be evaluated and adapted as required to meet the needs of the European Commission, and to tailor the level of detail, complexity and effort to fit the time and resources available. The quantitative methodology is flexible and can be adapted for different purposes. Introduction of plant pathogens is a consequence of the increase in plant trade over recent decades – one aspect of global change. The other perhaps longer-term aspect is climate change. It would be fruitful

to integrate the quantitative methodology with climate-change models to predict the future consequences of the introduction andof plant pathogens and arthropod pests into new territories.

Acknowledgements The authors thank all the Plant Health Panel and Working Group members, including Andy Hart and other external members, Hearing Experts, and EFSA staff involved in the EFSA Scientific Opinions mentioned in this Chapter. The position and opinions presented in this Chapter are those of the authors alone and do not necessarily represent the views of EFSA.

References

Anagnostakis, S. L., & Jaynes, R. A. (1973). Chestnut blight control: Use of hypovirulent cultures. *Plant Disease Reporter, 57*, 225–226.

Belli, G., Bianco, P. A., & Conti, M. (2010). Grapevine yellows in Italy: Past, present and future. *Journal of Plant Pathology, 92*, 303–326.

Bertin, S., Guglielmino, C. R., Karam, N., Gomulski, L. M., Malacrida, A. R., & Gasperi, G. (2007). Diffusion of the Nearctic leafhopper *Scaphoideus titanus* Ball in Europe: A consequence of human trading activity. *Genetica, 131*, 275–285.

Bianco, P. A., Fortusini, A., Scattini, G., Casati, P., Carraro, S., & Torresin, G. C. (2000). Experiments of heat treatment on dormant wood of grapevines affected by Flavescence doree. *Informatore Fitopatologico, 50*, 43–49.

Bonfils, J., & Schvester, D. (1960). Les cicadelles (Homoptera Auchenorhyncha) dans leurs rapports avec la vigne dans le Sud-Ouest de la France. *Annales des Epiphyties, 3*, 325–336.

Boyd, I. L., Freer-Smith, P. H., Gilligan, C. A., & Godfray, H. C. (2013). The consequence of tree pests and diseases for ecosystem services. *Science, 342*, 1235773.

Bragança, H., Simoes, S., Onofre, N., Tenreiro, R., & Rigling, D. (2007). *Cryphonectria parasitica* in Portugal: Diversity of vegetative compatibility types, mating types, and occurrence of hypovirulence. *Forest Pathology, 37*, 391–402.

Bressan, A., Larrue, J., & Boudon Padieu, E. (2006). Patterns of phytoplasma-infected and -infective *Scaphoideus titanus* leafhoppers in vineyards with high incidence of Flavescence dorée. *Entomologia Experimentalis et Applicata, 119*, 61–69.

Buonincontri, M. P., Saracino, A., & Di Pasquale, G. (2015). The transition of chestnut (*Castanea sativa* Miller) from timber to fruit tree: Cultural and economic inferences in the Italian peninsula. *The Holocene, 25*, 1111–1123.

Caudwell, A. (1990). Epidemiology and characterization of flavescence dorée (FD) and other grapevine yellows. *Agronomie, 10*, 655–663.

Caudwell, A., Larrue, J., Tassart, V., Boidron, R., Grenan, S., Leguay, M., & Bernard, P. (1994). Caractère porteur de la Flavescence dorée chez les vignes porte-greffes en particulier le 3309 C et le Fercal. *Agronomie, 14*, 83–94.

Caudwell, A., Larrue, J., Boudon-Padieu, E., & McLean, G. D. (1997). Flavescence dorée elimination from dormant wood of grapevines by hot-water treatment. *Australian Journal of Grape and Wine Research, 3*, 21–25.

Chuche, J., & Thiéry, D. (2014). Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: A review. *Agronomy for Sustainable Development, 34*, 381–403.

Colinas, C., & Uscuplic, M. (1999). Studies on chestnut blight (*Cryphonectria parasitica* (Murr.) Barr) in north-East Spain. *Acta Horticulturae, 494*, 495–500.

Cravedi, P., Mazzoni, E., & Cervato, P. (1993). Osservazioni sulla biologia di *Scaphoideus titanus* Ball (Homoptera: Cicadellidae). *Redia, 76*, 57–70.

Credi, R. (1989). Flavescenza dorata della vite in Emilia-Romagna: evoluzione della malattia nelle piante e suoi effetti sulla produzione e sullo sviluppo vegetativo. *Phytopathologia Mediterranea, 28*, 113–121.

Donatelli, M., Magarey, R. D., Bregaglio, S., Willocquet, L., Whish, J., & Savary, S. (2017). Modelling the impacts of pests and diseases on agricultural systems. *Agricultural Systems, 155*, 213–224.

Döring, T. F., Vieweger, A., Pautasso, M., Vaarst, M., Finckh, M. R., & Wolfe, M. S. (2015). Resilience as a universal criterion of health. *Journal of the Science of Food and Agriculture, 95*, 455–465.

Douma, J. C., Pautasso, M., Venette, R. C., Robinet, C., Hemerik, L., Mourits, M. C., Schans, J., & van der Werf, W. (2016). Pathway models for analysing and managing the introduction of alien plant pests an overview and categorization. *Ecological Modelling, 339*, 58–67.

EFSA. (2014). Guidance on expert knowledge elicitation in food and feed safety risk assessment. *EFSA Journal, 12*, 3734.

EFSA PLH Panel (EFSA Panel on Plant Health). (2014a). Scientific opinion on the pest categorisation of *Cryphonectria parasitica* (Murrill) Barr. *EFSA Journal, 12*, 3859.

EFSA PLH Panel (EFSA Panel on Plant Health). (2014b). Scientific opinion on the pest categorisation of *Diaporthe vaccinii* shear. *EFSA Journal, 12*, 3774.

EFSA PLH Panel (EFSA Panel on Plant Health). (2014c). Scientific opinion on the pest categorisation of *Ditylenchus destructor* Thome. *EFSA Journal, 12*, 3834.

EFSA PLH Panel (EFSA Panel on Plant Health). (2014d). Scientific opinion on pest categorisation of grapevine Flavescence Dorée. *EFSA Journal, 12*, 3851.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Urek, G., Van Bruggen, A., Van der Werf, W., West, J., Winter, S., Maresi, G., Prospero, S., Vettraino, A. M., Vloutoglou, I., Pautasso, M., & Rossi, V. (2016a). Scientific opinion on the risk assessment and reduction options for *Cryphonectria parasitica* in the EU. *EFSA Journal, 14*, 4641.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Gregoire, J.-C., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Van Bruggen, A., Van Der Werf, W., West, J., Winter, S., Mosbach-Schulz, O., & Urek, G. (2016b). Scientific opinion on the risk to plant health of *Ditylenchus destructor* for the EU territory. *EFSA Journal, 14*, 4602.

EFSA Panel on Plant Health (PLH), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Urek, G., Rossi, V., Van Bruggen, A., Van Der Werf, W., West, J., Winter, S., Bosco, D., Foissac, X., Strauss, G., Hollo, G., Mosbach-Schulz, O., & Grégoire, J.-C. (2016c). Scientific opinion on the risk to plant health of Flavescence dorée for the EU territory. *EFSA Journal, 14*, 4603.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Urek, G., Van Bruggen, A., Van der Werf, W., West, J., Winter, S., Santini, A., Tsopelas, P., Vloutoglou, I., Pautasso, M., & Rossi, V. (2016d). Scientific opinion on the risk assessment and reduction options for *Ceratocystis platani* in the EU. *EFSA Journal, 14*, 4640, 65 pp.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Gregoire, J.-C., Jaques Miret, J. A., MacLeod, A., Navarro, M. N., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Urek, G., Van Der Werf, W., West, J., Winter, S., Gardi, C., Mosbach-Schulz, O., Koufakis, I., & Van Bruggen, A. (2017a). Scientific opinion on the pest risk assessment of *Diaporthe vaccini* for the EU territory. *EFSA Journal, 15*, 4924.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Gregoire, J.-C., Jaques Miret, J. A.,

MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Van Bruggen, A., Van Der Werf, W., West, J., Winter, S., Schans, J., Kozelska, S., Mosbach-Schulz, O., & Urek, G. (2017b). Scientific opinion on the pest risk assessment of *Radopholus similis* for the EU territory. *EFSA Journal, 15*(4879), 265.

EFSA Panel on Plant Health (PLH), Jeger, M., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Gregoire, J.-C., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Urek, G., Van Bruggen, A., Van Der Werf, W., West, J., Winter, S., Boberg, J., Porta Puglia, A., Vettraino, A. M., Pautasso, M., & Rossi, V. (2017c). Scientific opinion on the pest risk assessment of *Atropellis* spp. for the EU territory. *EFSA Journal, 15*(4877), 46 pp.

EFSA PLH Panel (EFSA Panel on Plant Health), Jeger, M., Bragard, C., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gregoire, J.-C., Jaques Miret, J. A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Urek, G., Van Bruggen, A., Van Der Werf, W., West, J., Winter, S., Hart, A., Schans, J., Schrader, G., Suffert, M., Kertesz, V., Kozelska, S., Mannino, M. R., Mosbach-Schulz, O., Pautasso, M., Stancanelli, G., Tramontini, S., Vos, S., & Gilioli, G. (2018). Guidance on quantitative pest risk assessment. *EFSA Journal, 16*(5350), 86.

EPPO (European and Mediterranean Plant Protection Organization). (1997a). Data sheets on quarantine pests: *Diaporthe vaccinii*. Available online: https://gd.eppo.int/download/doc/101_datasheet_DIAPVA.pdf

EPPO (European and Mediterranean Plant Protection Organization). (1997b) Data sheets on quarantine pests: Grapevine flavescence dorée phytoplasma. Available online: https://gd.eppo.int/download/doc/192_datasheet_PHYP64.pdf

EPPO (European and Mediterranean Plant Protection Organization). (2015). First report of *Diaporthe vaccinii* in Poland and its subsequent eradication. *EPPO Reporting Service, 2015*(1), 3. https://gd.eppo.int/reporting/article-4358.

Eveillard, S., Jollard, C., Labroussaa, F., Khalil, D., Perrin, M., Desqué, D., Salar, P., Razan, F., Hévin, C., Bordenave, L., Foissac, X., Masson, J. E., & Malembic-Maher, S. (2016). Contrasting susceptibilities to Flavescence dorée in *Vitis vinifera*, rootstocks and wild Vitis species. *Frontiers in Plant Science, 7*, 1762.

Filippin, L., Jovic, J., Forte, V., Cvrkovic, T., Tosevski, I., Borgo, M., & Angelini, E. (2007). Occurrence and diversity of phytoplasmas detected in clematis and their relationships with grapevine "flavescence doree" phytoplasmas. *Bulletin of Insectology, 60*, 327–328.

Filippin, L., Jović, J., Cvrković, T., Forte, V., Clair, D., Toševski, I., Boudon-Padieu, E., Borgo, M., & Angelini, E. (2009). Molecular characteristics of phytoplasmas associated with Flavescence dorée in clematis and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector. *Plant Pathology, 58*, 826–837.

Freer-Smith, P. H., & Webber, J. F. (2017). Tree pests and diseases: The threat to biodiversity and the delivery of ecosystem services. *Biodiversity and Conservation, 26*, 3167–3181.

Gaffuri, F., Sacchi, S., & Cavagna, B. (2011). First detection of the mosaic leafhopper, *Orientus ishidae*, in northern Italian vineyards infected by the flavescence dorée phytoplasma. *New Disease Reports, 24*, 22.

Ghelardini, L., Luchi, N., Pecori, F., Pepori, A. L., Danti, R., Della Rocca, G., Capretti, P., Tsopelas, P., & Santini, A. (2017). Ecology of invasive forest pathogens. *Biological Invasions, 19*, 3183–3200.

Gilardi, G., Garibaldi, A., & Gullino, M. L. (2018). Emerging pathogens as a consequence of globalization and climate change: Leafy vegetables as a case study. *Phytopathologia Mediterranea, 57*, 146–152.

Gilioli, G., Schrader, G., Baker, R. H., Ceglarska, E., Kertész, V. K., Lövei, G., Navajas, M., Rossi, V., Tramontini, S., & Van Lenteren, J. C. (2014). Environmental risk assessment for plant pests: A procedure to evaluate their impacts on ecosystem services. *Science of the Total Environment, 468*, 475–486.

Gilioli, G., Schrader, G., Carlsson, N., van Donk, E., van Leeuwen, C. H., Martín, P. R., Pasquali, S., Vilà, M., & Vos, S. (2017a). Environmental risk assessment for invasive alien species:

A case study of apple snails affecting ecosystem services in Europe. *Environmental Impact Assessment Review, 65*, 1–11.

Gilioli, G., Schrader, G., Grégoire, J. C., MacLeod, A., Mosbach-Schulz, O., Rafoss, T., Rossi, V., Urek, G., & van der Werf, W. (2017b). The EFSA quantitative approach to pest risk assessment–methodological aspects and case studies. *EPPO Bulletin, 47*, 213–219.

Griffin, G. J. (1986). Chestnut blight and its control. *Horticultural Reviews, 8*, 291–335.

Gusberti, M., Jermini, M., Wyss, E., & Linder, C. (2008). Efficacité d'insecticides contre *Scaphoideus titanus* en vignobles biologiques et effets secondaires. *Revie Suisse de Viticulture, Arboriculture et Horticulture, 40*, 173–177.

Heiniger, U., & Rigling, D. (1994). Biological control of chestnut blight in Europe. *Annual Review of Phytopathology, 32*, 581–599.

Heiniger, U., & Rigling, D. (2009). Application of the Cryphonectria hypovirus (CHV-1) to control the chestnut blight, experience from Switzerland. *Acta Horticulturae, 815*, 233–245.

Hijmans, R. J. (2001). Global distribution of the potato crop. *American Journal of Potato Research, 78*, 403–412.

Holt, J., Leach, A. W., MacLeod, A., Tomlinson, D., Christodoulou, M., & Mumford, J. D. (2017). A quantitative model for trade pathway analysis of plant pest entry and transfer to a host in European Union territory. *EPPO Bulletin, 47*, 220–226.

Ježić, M., Krstin, L., Rigling, D., & Ćurković-Perica, M. (2012). High diversity in populations of the introduced plant pathogen, *Cryphonectria parasitica*, due to encounters of genetically divergent genotypes. *Molecular Ecology, 21*, 87–99.

Jones, G. (2018). The economic analysis of plant health and the needs of policy makers. In J. Urquhart, M. Marzano, & C. Potter (Eds.), *The human dimensions of Forest and tree health* (pp. 211–234). Berlin: Springer.

Landolt, J., Gross, A., Holdenrieder, O., & Pautasso, M. (2016). Ash dieback due to *Hymenoscyphus fraxineus*: What can be learnt from evolutionary ecology? *Plant Pathology, 65*, 1056–1070.

Lessio, F., Tedeschi, R., & Alma, A. (2007). Presence of *Scaphoideus titanus* on American grapevine in woodlands, and infection with "flavescence dorée" phytoplasmas. *Bulletin of Insectology, 60*, 373–374.

Lessio, F., Tota, F., & Alma, A. (2014). Tracking the dispersion of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) from wild to cultivated grapevine: Use of a novel mark–capture technique. *Bulletin of Entomological Research, 104*, 432–443.

Linder, C., Schaub, L., & Klotzli-Estermann, F. (2010). Effectiveness of hot water treatment against the eggs of *Scaphoideus titanus* Ball, vector of the grapevine flavescence doree. *Revue Suisse de Viticulture, Arboriculture et Horticulture, 42*, 132–135.

Lombard, L., van Leeuwen, G. C. M., Guarnaccia, V., Polizzi, G., van Rijswijk, P. C. J., Rosendahl, C. H. M., Gabler, J., & Crous, P. W. (2014). *Diaporthe* species associated with *Vaccinium*, with specific reference to Europe. *Phytopathologia Mediterranea, 53*, 287–299.

MacGuidwin, A. E., Wixted, D. J., & Hudelson, B. D. (1992). Aboveground infection of snap bean by *Ditylenchus destructor*, the potato rot nematode. *Plant Disease, 76*, 1097–1102.

Malembic, S., Salar, P., Vergnes, D., & Foissac, X. (2007). Detection and diversity of "flavescence dorée"-related phytoplasmas in alders surrounding infected vineyards in Aquitaine (France). *Bulletin of Insectology, 60*, 329–330.

Mannini, F., & Marzachì, C. (2007). Termoterapia in acqua contro i fitoplasmi della vite. *Informatore Agrario, 24*, 62–65.

Mattioni, C., Martin, M. A., Pollegioni, P., Cherubini, M., & Villani, F. (2013). Microsatellite markers reveal a strong geographical structure in European populations of *Castanea sativa* (Fagaceae): Evidence for multiple glacial refugia. *American Journal of Botany, 100*, 951–961.

McMullan, M., Rafiqi, M., Kaithakottil, G., Clavijo, B. J., Bilham, L., Orton, E., Percival-Alwyn, L., Ward, B. J., Edwards, A., Saunders, D. G., & Accinelli, G. G. (2018). The ash dieback invasion of Europe was founded by two genetically divergent individuals. *Nature Ecology & Evolution, 2*, 1000–1008.

Mehle, N., Seljak, G., Rupar, M., Ravnikar, M., & Dermastia, M. (2010). The first detection of a phytoplasma from the 16SrV (Elm yellows) group in the mosaic leafhopper *Orientus ishidae*. *New Disease Reports, 22*, 11.

Mellano, M. G., Beccaro, G. L., Donno, D., Marinoni, D. T., Boccacci, P., Canterino, S., Cerutti, A. K., & Bounous, G. (2012). *Castanea* spp. biodiversity conservation: Collection and characterization of the genetic diversity of an endangered species. *Genetic Resources and Crop Evolution, 59*, 1727–1741.

Michalecka, M., Bryk, H., & Seliga, P. (2017). Identification and characterization of *Diaporthe vaccinii* shear causing upright dieback and viscid rot of cranberry in Poland. *European Journal of Plant Pathology, 148*, 595–605.

Milgroom, M. G., & Cortesi, P. (2004). Biological control of chestnut blight with hypovirulence: A critical analysis. *Annual Review of Phytopathology, 42*, 311–338.

Morone, C., Gotta, P., & Marzachì, C. (2001). Riconoscimento dei sintomi di inizio stagione della flavescenza dorata. *Informatore Agrario, 12*, 83–86.

Morone, C., Boveri, M., Giosuè Gotta, P., Rossi, V., Scapin, I., & Marzachì, C. (2007). Epidemiology of Flavescence dorée in vineyards in northwestern Italy. *Phytopathology, 97*, 1422–1427.

Moutous, G. (1977). Definition of golden flavescence symptoms on some vine-stocks. *Revue de Zoologie Agricole et de Pathologie Végetale, 76*, 90–98.

Mwaura, P., Niere, B., & Vidal, S. (2014). Resistance and tolerance of potato varieties to potato rot nematode (*Ditylenchus destructor*) and stem nematode (*Ditylenchus dipsaci*). *Annals of Applied Biology, 166*, 1–14.

Mwaura, P., Niere, B., & Vidal, S. (2015). Effect of initial population densities of *Ditylenchus destructor* and *D. dipsaci* on potato tuber damage and nematode reproduction. *Nematology, 17*, 193–202.

Narouei-Khandan, H. A., Harmon Lapaire, C., Harmon, P., Olmstead, J., Zelenev, V. V., dervan Werf, W., Worner, S. P., Senay, S. D., & van AHC, B. (2017). Potential global and regional geographic distribution of *Phomopsis vaccinii* on *Vaccinium* species projected by two species distribution models. *European Journal of Plant Pathology, 148*, 919–930.

Oerke, E. C. (2006). Crop losses to pests. *The Journal of Agricultural Science, 144*, 31–43.

Olatinwo, R. O., Hanson, E. J., & Schilder, A. M. C. (2004). Incidence and causes of postharvest fruit rot in stored Michigan cranberries. *Plant Disease, 88*, 1277–1282.

Pavan, F., Mori, N., Bigot, G., & Zandigiacomo, P. (2012a). Border effect in spatial distribution of Flavescence dorée affected grapevines and outside source of *Scaphoideus titanus* vectors. *Bulletin of Insectology, 65*, 281–290.

Pavan, F., Mori, N., Bressan, S., & Mutton, P. (2012b). Control strategies for grapevine phytoplasma diseases: Factors influencing the profitability of replacing symptomatic plants. *Phytopathologia Mediterranea, 51*, 11–22.

Pezzi, G., Maresi, G., Conedera, M., & Ferrari, C. (2011). Woody species composition of chestnut stands in the northern Apennines: The result of 200 years of changes in land use. *Landscape Ecology, 26*, 1463–1476.

Polashock, J. J., & Kramer, M. (2006). Resistance of blueberry cultivars to Botryosphaeria stem blight and Phomopsis twig blight. *Horticultural Science, 41*, 1457–1461.

Prospero, S., & Cleary, M. (2017). Effects of host variability on the spread of invasive forest diseases. *Forests, 8*, 80.

Prospero, S., & Rigling, D. (2016). Using molecular markers to assess the establishment and spread of a mycovirus applied as a biological control agent against chestnut blight. *BioControl, 61*, 313–323.

Pueyo, C., Carrara, J. & Parent, E. (2008). *Flavescence dorée en Languedoc-Roussillon: bilan de 10 années de lutte (synthèse des données 1997–2007)*. Direction Régionale de l'Agriculture et de la Forêt Languedoc-Roussillon, Service Régional de la Protection des Végétaux, Montpellier, France. 21 pp. Available online: http://www.vignevin.com/fileadmin/users/ifv/actualites/Lettre_Oct12/DocSite/Flavescence/10_ans_de_lutte_contre_la_flavescence1a.pdf

Rigling, D., & Prospero, S. (2018). *Cryphonectria parasitica*, the causal agent of chestnut blight: Invasion history, population biology and disease control. *Molecular Plant Pathology, 19*, 7–20.

Rimbaud, L., et al. (2018). Using sensitivity analysis to identify key factors for the propagation of a plant epidemic. *Royal Society Open Science, 5*, 171435.

Roggia, C., Caciagli, P., Galetto, L., Pacifico, D., Veratti, F., Bosco, D., & Marzachì, C. (2014). Flavescence doree phytoplasma titre in field-infected Barbera and Nebbiolo grapevines. *Plant Pathology, 63*, 31–41.

Sabaratnam, S., Wood, B., & Nabetani, K. (2016). *Fruit rot pathogens and their impact on cranberry production in British Columbia [2014 study]* (14 pp). Abbotsford: Abbotsford Agriculture Centre, Ministry of Agriculture.

Savary, S., Ficke, A., Aubertot, J. N., & Hollier, C. (2012). Crop losses due to diseases and their implications for global food production losses and food security. *Food Security, 4*, 519–537.

Savary, S., Nelson, A. D., Djurle, A., Esker, P. D., Sparks, S., Amorim, L., Bergamin Filho, A., Caffi, T., Castilla, N., Garrett, K., McRoberts, N., Rossi, V., Yuen, J., & Willocquet, L. (2018). Concepts, approaches, and avenues for modelling crop health and crop losses. *European Journal of Agronomy, 100*, 4–18.

Schvester, D., Carle, P., & Moutous, G. (1967). Essais de sensibilité de cépages à la flavescence dorée par inoculation avec *Scaphoideus littoralis* Ball. *Annales des Epiphyties, 18*, 143–150.

Seidl, R., Klonner, G., Rammer, W., Essl, F., Moreno, A., Neumann, M., & Dullinger, S. (2018). Invasive alien pests threaten the carbon stored in Europe's forests. *Nature Communications, 9*, 1626.

Sicard, A., Zeilinger, A. R., Vanhove, M., Schartel, T. E., Beal, D. J., Daugherty, M. P., & Almeida, R. P. (2018). *Xylella fastidiosa*: Insights into an emerging plant pathogen. *Annual Review of Phytopathology, 56*, 181–202.

Sivčev, B. V., Sivčev, I. L., & Vasic, Z. Z. R. (2010). Plant protection products in organic grapevine growing. *Journal of Agricultural Sciences, Belgrade, 55*, 103–122. https://doi.org/10.2298/jas1001103s.

Sofaer, H. R., Jarnevich, C. S., & Pearse, I. S. (2018). The relationship between invader abundance and impact. *Ecosphere, 9*(9), e02415.

Stiles, C. M., & Oudemans, P. V. (1999). Distribution of cranberry fruit-rotting fungi in New Jersey and evidence for nonspecific host resistance. *Phytopathology, 89*, 218–225.

Strømeng, G. M., & Stensvand, A. (2011). Godronia Canker (*Godronia cassandrae* f. sp. vaccinii) in highbush blueberry. *The European Journal of Plant Science and Biotechnology, 5*(S1), 35–41.

Sturhan, D., & Brzeski, M. W. (1991). Stem and bulb nematodes, *Ditylenchus* spp. In W. R. Nickle (Ed.), *Manual of agricultural nematology* (pp. 423–464). New York: Marcel Dekker.

Suffert, M., Wilstermann, A., Petter, F., Schrader, G., & Grousset, F. (2018). Identification of new pests likely to be introduced into Europe with the fruit trade. *EPPO Bulletin, 48*, 144–154.

Tothova, M., Bokor, P., & Cagan, Ľ. (2015). The first detection of leafhopper *Scaphoideus titanus* Ball (Hemiptera, Cicadellidae) in Slovakia. *Plant Protection Science, 51*, 88–93.

Trivellone, V., Filippin, L., Narduzzi-Wicht, B., & Angelini, E. (2016). A regional-scale survey to define the known and potential vectors of grapevine yellow phytoplasmas in vineyards south of Swiss Alps. *European Journal of Plant Pathology, 145*, 915–927.

Turchetti, T., & Maresi, G. (2008). Biological control and management of chestnut diseases. In A. Ciancio & K. G. Mukerji (Eds.), *Integrated management of diseases caused by fungi, phytoplasma and bacteria* (pp. 85–118). Dordrecht: Springer.

Valenta, V., Gilioli, G., & Schrader, G. (2017). Umweltrisikoanalyse des Asiatischen Eschenprachtkäfers *Agrilus planipennis* Fairmaire für Europa. *Journal für Kulturpflanzen, 69*, 1–9.

van Bruggen, A. H. C., West, J. S., van der Werf, W., Potting, R. P. J., Gardi, C., Koufakis, I., Zelenev, V. V., Narouei-Khandan, H., Schilder, A., & Harmon, P. (2018). Input data needed for a risk model for the entry, establishment and spread of a pathogen (Phomopsis vaccinii) of blueberries and cranberries in the EU. *Annals of Applied Biology, 172*, 126–147.

Vilka, L., & Volkova, J. (2015). Morphological diversity of *Phomopsis vaccinii* isolates from cranberry (*Vaccinium macrocarpon* Ait.) in Latvia. *Proceedings of the Latvia University of Agriculture, 33*, 8–18.

Vilka, L., Rancane, R., & Eihe, M. (2009). Storage rots of Vaccinium macrocarpon spread and development in Latvia. *Latvia Agronomijas Vestis, 12*, 133–137.

Weingartner, D. P., & Klos, E. J. (1975). Etiology and symptomatology of canker and dieback diseases on highbush blueberries caused by *Godronia (Fusicoccum) cassandrae* and *Diaporthe (Phomopsis) vaccinii*. *Phytopathology, 65*, 105–110.

Weintraub, P. G., & Beanland, L. (2006). Insect vectors of phytoplasmas. *Annual Review of Entomology, 51*, 91–111.

Whitehead, A. G. (1998). *Plant Nematode Control* (384 pp). Wallingford: CAB International.

Part IV
Innovative Techniques for Monitoring Emerging Diseases

Chapter 9
Diagnosis and Assessment of Some Fungal Pathogens of Rice: Novel Methods Bring New Opportunities

Sara Franco Ortega, Davide Spadaro, and Maria Lodovica Gullino

Abstract Global food security depends to a substantial degree on the rice crop. Rice production is threatened by many pathogens, requiring a range of prevention and control strategies. These need to be integrated with efficient monitoring and detection. This chapter focuses on diagnostic tools developed for three of the most important rice pathogens: *Pyricularia oryzae*, the agent of rice blast; *Bipolaris oryzae*, the agent of rice brown spot; and *Fusarium fujikuroi*, the agent of bakanae disease. Accurate, effective, sensitive and specific diagnostic techniques are required to forecast and manage these diseases. Observation of symptoms in field crops is not always accurate. Serological and, especially, molecular techniques present advantages in terms of speed, specificity and ease of use. Whole-genome sequencing has revealed novel genomic regions and genes and can be used to distinguish between clonal lineages of pathogens. High-throughput sequencing applied to the aerial mycobiome of rice crops has revealed substantial biodiversity that can affect the airborne inoculum and may play a key role in survival and dispersal of fungal spores. The LAMP (Loop-mediated isothermal AMPlification) assay has been identified as a suitable solution for on-site detection. The application of LAMP combined with alkaline DNA extraction has an enormous potential to assess airborne and other inoculum. The use of molecular field testing is revolutionising disease diagnostics, giving results in a few hours. There are exciting prospects of further

S. Franco Ortega
Centre of Competence for the Innovation in the Agro-environmental Sector – AGROINNOVA, University of Turin, Grugliasco, TO, Italy

D. Spadaro (✉)
Centre of Competence for the Innovation in the Agro-environmental Sector – AGROINNOVA, University of Turin, Grugliasco, TO, Italy

Department of Agricultural, Forestry and Food Sciences (DiSAFA), University of Torino, Grugliasco, TO, Italy
e-mail: davide.spadaro@unito.it

M. L. Gullino
Università di Torino, Centro Agroinnova, Grugliasco, Italy
e-mail: marialodovica.gullino@unito.it

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_9

advances through machine learning. Improved detection should lead to better disease control and hence improved food security. However, pathogens are genetically dynamic so plant disease management will remain challenging.

Introduction

An increase of 3 billion in the world population, reaching between 8.9 and 10.0 billion people, is expected by 2050 (Carvalho 2006; United Nations 2015). Inhabitants of South-East Asia base their daily caloric intake (50 to 80%) on rice (Delseny et al. 2001). As a consequence, rice production needs to increase from 439 million tons in 2010 to an estimated 555 million tons in 2035 (Gathala et al. 2011). This is a challenging goal, given the low availability of farmland due to industrialization and urbanization, and because of climate change and the occurrence of new plant diseases. Appropriate management of the rice crop has thus assumed global importance.

Rice (*Oryza* spp.) is a monocotyledonous annual grass, which includes 24 species divided into nine diploid and three tetraploid genome types (Ge et al. 1999; Nayar 2014). The most important cultivars belong to the cultivated species *O. sativa* and *O. glaberrima,* the latter being grown only in Africa. In terms of production, rice is the second most important cereal after maize, with more than 750 million tons produced in 2017, from 114 producer countries around the world. Asian countries consume 90% of the rice produced (http://ricestat.irri.org:8080/wrsv3/entrypoint.htm).

Rice production has doubled since the 1960s thanks to continuous improvements in cultivation practices and the introduction of high-yielding varieties, including semi-dwarf varieties (Khush 2005). Still greater production is hampered by the limited germplasm available and by the biotic and abiotic stresses, which can cause up to 30% yield losses (Skamnioti and Gurr 2009). Rice production is limited by numerous diseases, many of them affecting all rice-growing countries; the challenge is to use disease control strategies that can avoid major yield losses. Some diseases cause acute outbreaks in limited areas, with more or less severe consequences (Savary et al. 2011a, b). Most rice infections, in the field and during storage, are caused by fungi. The most damaging pathogens include *Pyricularia oryzae* causing rice blast (RB), *Bipolaris oryzae* causing brown leaf spot, *Curvularia* spp. causing blight-related symptoms, *Alternaria padwickii* causing stackburn, *Cercospora jansena* causing narrow brown spot (BS), *Rhizoctonia solani* causing sheath blight, and *Sarocladium oryzae* causing sheath rot.

In addition, many mycotoxigenic species of *Fusarium*, *Penicillium* and *Aspergillus* are able to cause mycotoxin contamination of rice grains, threatening food security and presenting a risk to animal and human health (Tanaka et al. 2007). Furthermore, *Fusarium* spp. can damage rice in several different ways, such as root-rot, seed-rot, and seedling damage; they are also the cause of bakanae disease (Almaguer et al. 2008).

Sharma and Thind (2007) reported that RB, sheath blight, BS, sheath rot and bakanae are the five most important rice diseases in India. Appropriate management

of RB and bakanae is becoming crucial, due to the high intra-species genetic variation of the pathogens, and due to their seed transmission (Mew and Gonzales 2002; Gupta et al. 2014).

Rice seeds are an important entry pathway for some diseases. This is of particular concern for smallholder farmers who save their own seed (Fujisaka et al. 1993; Joshi et al. 2000). Over 100 fungal species have been identified as seedborne or seed-transmitted. Movement of infected seeds may establish a pathogen in a new area (Bishaw et al. 2012).

New management strategies are needed to address the challenge to food security of a changing pattern of rice diseases. Rapid and sensitive diagnosis of pathogens is a critical requirement for effective control of disease outbreaks and to prevent spread into new areas. This chapter focuses on diagnostic and assay techniques for three globally important rice diseases as examples, with particular attention to the development of molecular techniques.

Rice Blast, One of the Most Destructive Rice Diseases

P. oryzae Cavara is the causal agent of grey leaf spot and RB. It occurs worldwide in over 50 grass species, including rice, wheat, barley, maize, oats, rye, finger millet, perennial ryegrass, weed and ornamental grasses (Ou 1985). RB causes major losses in all rice-growing regions (Talbot 2003); it is responsible for 10–30% losses annually in India (Khush and Jena 2009). It is anticipated that losses may increase with global warming. One of the critical problems in the management of RB is that *P. oryzae* is endemic in all rice-growing countries. The lack of varieties resistant to RB in the main producer countries, accompanied by the excessive use of nitrogen fertilizers, makes its management critical. *P. oryzae* is able to infect all parts of the plant including leaves, collar, neck and panicle.

Host Specificity of Isolates

The majority of *P. oryzae* isolates are host-specific. However, while *P. oryzae* isolates from species of *Eleusine*, *Setaria* and *Triticum* are not able to infect rice, some *P. oryzae* isolates from rice are able to infect a few other species such as *Hordeum* spp. or *Lolium* spp. (Kato et al. 2000; Tosa and Chuma 2014). *P. oryzae* isolates from other grasses and *P. grisea* from *Digitaria sanguinalis* are able to cross-infect between host species (Choi et al. 2013). Given such variation in host-specificity, the host species on which a *Pyricularia* isolate is observed is not a definitive criterion for assigning it to a species. Indeed, *P. grisea* and *P. oryzae* have been treated as alternative synonyms, *P. oryzae* being applied to isolates from rice and *P. grisea* to isolates from other host species (Sprague 1950). Because mating between isolates from different hosts was found to be unsuccessful, the two species have been considered as single-biological taxonomic units (Hebert 1971; Kato et al. 2000).

See under molecular methods for further aspects of characterization of *Pyricularia* isolates.

In-field Monitoring by Visual Observation and Spore Trapping

The aggressiveness of the pathogen and its ability to cause a polycyclic epidemic can result in severe outbreaks if timely control measures are not applied. Early identification and detection of *P. oryzae* is essential for effective intervention. In the field, diagnosis is typically based on visual recognition of symptoms using a 0 to 9 scale that follows the different stages of the disease (IRRI 1988).

The primary inoculum (ascospores) and the secondary inoculum (conidia) are airborne. Monitoring the inoculum may help to determine the most appropriate management strategies. Spore trapping devices are widely used, the most popular type being the Hirst spore trap (Hirst 1954), in which spores impact onto sticky tapes on rotating drums, and can be counted weekly by microscopic observation. The conidia of *P. oryzae* are recognized by their hyaline or light olive-green colour and their pear-like shape with two septa and a basal appendage. The results of monitoring airborne inoculum, together with meteorological data, can be used to forecast the course of the disease, allowing appropriate management strategies to be deployed, such as the application of fungicides. Unfortunately the monitoring process is time-consuming and may not be accurate, due to the high concentrations of spores at some points and the difficulty of distinguishing spores of *P. oryzae* from those of other species.

See under PCR-based seed testing and field monitoring for further aspects.

Seed Testing

Seed transmission of *P. oryzae* was initially described by Kuribayashi (1928). Seeds and infested debris are an important reservoir of the pathogen and may be the primary inoculum source (Faivre-Rampant et al. 2013). New identification techniques and the occurrence of new alien species have boosted the development of seed health testing (Chadha and Gopalakrishna 2006). Certified laboratories and seed-testing companies follow international quality standards and protocols to declare the absence of rice pathogens in seeds. The International Rules for Seed Testing are approved by the International Seed Testing Association (ISTA).

Identification of *P. oryzae* in rice seeds has normally been performed by macroscopic and microscopic observation (Neergaard et al. 1970; ISTA 2019). In the "blotting test" a sample of 400 rice seeds is incubated in petri dishes for 7 days at 22 °C with alternating light and darkness. Seeds are then examined under a stereoscopic microscope to detect the characteristic conidia. The fungus is often found on the pedicels.

Such methods are time-consuming and laborious, and require an expert for morphological identification of the conidia. Typically, saprophytic fungi on the seed surface overgrow the pathogen so its prevalence is underestimated (Mathur 1995; Atkins and Clark 2004). *P. oryzae* cannot be distinguished from *P. grisea*, even if perithecia and ascospores are also observed (Kato et al. 2000; Couch and Kohn 2002; Choi et al. 2013).

See under PCR-based seed testing for further aspects.

Serological Methods

To overcome the problems of recognition, methods have been developed to detect *P. oryzae* using polyclonal (Xia et al. 1992) or monoclonal (Lee et al. 1998; Hasegawa et al. 1999) antibodies. Through Enzyme-Linked Immunosorbent Assay (ELISA) it was possible to detect 10–20 conidia per well with polyclonal antibodies, and 0.1μg per 100 mg of fresh rice leaf tissue with monoclonal antibodies. Immunological techniques have also been used for early detection (Schaad et al. 2003). However, such methods have limitations associated with the low immunological response to fungal antigens in mammals (Ward et al. 1998).

Molecular Methods

Genome-based molecular methods address some of the problems of identification by traditional and serological methods. These methods involve amplification of a region of the fungal genome to design specific primers. They include the use of Rapid Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP), Quantitative PCR (qPCR) and Repetitive Element-based Polymerase Chain Reaction (rep-PCR) (George et al. 1998; Koizumi et al. 2004; Motallebi et al. 2009).

Genome-based methods were originally based on primers specific for the identification of *P. oryzae* pathotypes (Atkins and Clark 2004; Chadha and Gopalakrishna 2006). Hamer et al. (1989), using amplification of the repetitive element *Pot2,* present in over 100 copies in *P. grisea*, could distinguish rice-infecting isolates from isolates infecting other grasses. Use of REMAP based on MAGGY (a gypsy-like LTR retrotransposon identified in *P. grisea*) (Nakayashiki et al. 1999) also allowed useful characterization of population structure (Chadha and Gopalakrishna 2005). Couch and Kohn (2002) investigated the phylogenetic relationships between *P. grisea* isolates from different hosts by Multilocus Sequence Analysis (MLSA), to determine whether synonymized isolates belonged to paraphyletic, polyphyletic or monophyletic clades. In *P. oryzae*, the structure of rRNA has been explored (Sone

et al. 2000) and has been used to study variability between isolates from rice fields in India (Jagadeesh et al. 2018).

Combining molecular data from ribosomal RNA, mitochondrial DNA and nuclear DNA, Borromeo et al. (1993) distinguished four main groups of *Pyricularia* isolates. Data from actin, beta-tubulin and calmodulin genes indicated two distinct monophyletic groups, one from *Digitaria* isolates and the other from *Oryza sativa* isolates (Couch and Kohn 2002). Klaubauf et al. (2014) used MLSA of genes for actin, RNA polymerase II subunit 1(RPB1), calmodulin, and two regions of ribosomal RNA.

Whole-genome sequencing has been used to distinguish between clonal lineages of *P. oryzae* that present extensive genome variation (Farman et al. 2005). Virulence effectors have been characterized (Chen et al. 2013). The genome of *P. oryzae* from a hybrid of rice and weeping lovegrass (Leung et al. 1988) has been sequenced with Sanger technology (Dean et al. 2005). Other isolates have been sequenced with high-throughput methods such as 454 technology (Xue et al. 2012) and the Illumina sequencing technology (Chen et al. 2013; Gowda et al. 2015).

PCR-based Seed Testing

A wide range of PCR-based analyses have been designed and tested to identify *P. oryzae*. Chadha and Gopalakrishna (2006) based a PCR on the infection-specific gene *mif-23* of *P. oryzae,* testing it on genetically and geographically diverse isolates. It could detect the fungus in rice seeds at infection rates of 0.2%. Use of qPCR instead of PCR allowed detection of even one fungal cell. A TaqMan qPCR assay was based on the Hydrophobin class 1 *(MHP1)* gene that is required for surface hydrophobicity, pathogenicity and infection of *M. oryzae* (Su'udi et al. 2013).

While qPCR remains the gold-standard technique for quantification, it may provide inaccurate results due to inadequate dilution of the samples for absolute quantification through the standard curve, or due to inhibition of the Taq polymerase by the presence of residual proteins or other molecules after DNA extraction (Taylor et al. 2017). Even greater sensitivity and precision is achieved by Digital Droplet PCR (ddPCR) (Manandhar and Jorgensen 1998), which can detect a single copy of a gene. This may be the next step in development of targeted quantification techniques (Hindson et al. 2011, 2013).

PCR-based Field Monitoring

The application of PCR-based analysis to sticky tape samples has been tested on other pathogens, such as *Monilinia fructicola* (Luo et al. 2007) and *Venturia inaequalis* (Meitz-Hopkins et al. 2014; Huang et al. 2016), and has also been applied to *P. oryzae* (Huang et al. 2016). High-throughput sequencing applied to the aerial

mycobiome of rice has revealed great biodiversity in the airborne ecosystem (Franco Ortega et al. 2020; Piombo et al 2021), which may affect the survival and dispersal of *P. oryzae* spores. The use of barcodes with greater resolving power for the genus *Pyricularia*, or the application of novel computational techniques such as oligotyping (Eren et al. 2013) to assign individuals to groups showing similarities below the level of operational taxonomic unit (OTU), may reveal patterns in fungal distribution, for example correlation with meteorological data. This could allow development of new models for RB management.

Rice seed infested with *P. oryzae* usually shows brown spots, blotches or occasionally diamond-shaped lesions, but they may not be easily recognized. Mistaken or failed recognition may cause the spread of RB to new areas, or the movement of genetically different lineages. In commercial seed trading, cost-effective methods are needed to certify samples as free from *P. oryzae*. They should be suitable for use by non-experts in molecular biology and should avoid false negatives and false positives.

The Loop-Mediated Isothermal AMPlification (LAMP) assay has been recognized as suitable for on-site detection. Initially described by Notomi et al. (2000) and later enhanced by Nagamine et al. (2002) it is based on isothermal amplification (optimally at 65 °C) of a target region by means of 4 or 6 primers that recognize 6 or 8 regions. Its sensitivity, in the range of picograms of DNA, and its accuracy has prompted the design and validation of new assays to detect other pathogens. A LAMP assay for *P. oryzae* based on the calmodulin gene has been designed and validated to international standard EPPO 7/98 for testing rice seeds (Franco Ortega et al. 2018). LAMP assay combined with alkaline DNA extraction (Tomlinson et al. 2010) has great potential for assessing airborne inoculum of *P. oryzae*, captured by spore traps (Villari et al. 2017).

Bipolaris oryzae, the Brown Spot Pathogen, Cause of the Great Bengal Famine

Bipolaris oryzae (Breda de Haan) Shoemaker, formerly *Helminthosporium oryzae,* is the causal agent of rice brown spot (BS) affecting millions of hectares worldwide (Ohata 1989; Chakrabarti et al. 1992; Chakrabarti 2001; Zanão Júnior et al. 2009; Savary et al. 2012)**.** It has caused two historically important epidemics: the first in 1918–1919 in Krishna-Godavari (Chakrabarti 2001); the second in 1942, when several outbreaks on the winter rice crop in India and Bangladesh caused the Great Bengal Famine (Padmanabhan 1973) with consequences that have been compared with the Irish potato famine in 1845. BS has been reported as one of the most destructive rice diseases, its high incidence and the aggressiveness of some strains causing major yield losses in all rice-growing countries (Savary et al. 2005). In tropical and subtropical Asia it causes losses ranging from 4 to 52% (Aluko 1975; Savary et al. 2006), up to 67% in some areas (Kohls et al. 1987), and reaching

75–100% (Percich et al. 1997). The disease is often a problem for resource-poor farmers, being favoured by low nitrogen fertilization (Ou 1985; Savary et al. 1997). The effects of global warming on BS development are still unknown.

The pathogen overwinters in debris, soil and some weeds (Ou 1985; Biswas et al. 2008; Sato et al. 2008). It causes a polycyclic disease with an important airborne phase (Johnson and Percich 1992).

BS epidemics are favoured by drought, and by seasons with moderate rainfall and increased relative humidity Plant age is critical (Padmanabhan and Ganguly 1954) and severe disease symptoms have been reported during ripening. Adverse effects of the disease include reduced tiller number, reduced leaf area and early senescence (Vidhyasekharan et al. 1973).

The genome of *B. oryzae* has been sequenced (Condon et al. 2013). It is thought that the fungus causes rapid cell death through non-specific phytotoxins (Ahn et al. 2005).

In-field Monitoring and Seed Testing

Diagnosis of BS in the field is by naked-eye observation utilizing a 0 to 9 scale that registers the different stages of the disease (IRRI 1988). BS outbreaks are often associated with infected seeds (Ellis and Holliday 1971; Nyvall 1995; Ba and Sangchote 2006). If the grain is infected, its quality decreases and germination may be reduced (Padmanabhan 1977). Accurate diagnosis and appropriate seed treatment is critical for effective management of seedborne infection (Biswas et al. 2008; Barnwal et al. 2013). The presence of *B. oryzae* in seeds can be detected by blotting tests in which 400 seeds are incubated at 23 ± 2 °C under 12 h light/12 h dark conditions (Brasil 2009; Moura et al. 2014). After 7 days, seeds are examined microscopically for the large, gently curving, canoe-shaped conidia and conidiophores of *B. oryzae*. The procedure is laborious and requires expertise; even experienced workers may deliver differing results.

Molecular Characterization

Molecular tools have revealed a significant degree of intra-species nucleotide variation in *B. oryzae*. This molecular characterization has been based on fingerprinting of microsatellite DNA (Burgos et al. 2013), universal rice primer (URP) markers (Banerjee et al. 2014). Phylogenetic analysis has been based on the ITS region, glyceraldehyde-3-phosphate dehydrogenase gene, elongation factor 1-alpha, and the large subunit rRNA (Berbee et al. 1999; Olivier et al. 2000; Dela Paz et al. 2006; Manamgoda et al. 2012). If such variation proves to be associated with variation in

virulence or fungicide resistance, molecular techniques could contribute to improved management of the disease.

PCR-based Monitoring

For the quantitative assessment of *B. oryzae,* Su'udi et al. (2012) developed a TaqMan real-time assay based on the scytalone dehydratase 1 (CmSCD1) gene, which is a single-copy gene necessary for melanin biosynthesis. This has been used to evaluate BS in rice leaf tissue and to evaluate airborne inoculum of *B. oryzae* during the rice cropping season, with reference to a metabarcoding and oligotyping analysis of the genus (Franco Ortega et al. 2020; Piombo et al. 2021). Such studies may be useful as a basis for disease forecasting systems (Barnwal et al. 2013).

Fusarium fujikuroi, a Soilborne and Seedborne Fungus, Causal Agent of Bakanae

Bakanae disease may be caused by several *Fusarium* species belonging to the polyphyletic *Gibberella fujikuroi* species complex (GFSC) (Wulff et al. 2010). *Fusarium fujikuroi* Nirenberg [teleomorph *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura] is the one mainly responsible (Amatulli et al. 2010). The fungus was initially described as *Lisea fujikuroi* (Sawada 1919); it was renamed *Gibberella fujikuroi* (Sun and Snyder 1981). It is in the GFSC section Liseola (O'Donnell and Cigelnik 1997; O'Donnell et al. 1998).

Bakanae is one of the most important diseases of rice in all rice-growing countries, causing losses up to 15% (Ito and Kimura 1931; Sharma and Thind 2007; Mohd Zainudin et al. 2008). Bakanae (the Japanese word means foolish seedling) is characterized by abnormal elongation of the seedling ("thin noodle seedling") (Sun and Snyder 1981), caused by production of gibberellin and abscisic acid and inhibition of jasmonic acid and phytoalexins (Siciliano et al. 2015). Other symptoms are seedling blight, root rot, grain discoloration, reduced germination and crown rot (Sun and Snyder 1981; Ou 1985; Wulff et al. 2010).

Unlike rice blast and brown spot, bakanae is a monocyclic disease. The fungus overwinters in infected seeds (the main inoculum source) or in the soil. Healthy seeds sown in infected soil result in infected seedlings, with bakanae symptoms if the inoculum is moderate or with symptoms of seedling blight if it is high (Cartwright et al. 2018). Environmental conditions play a key role in symptom development: at temperatures up to 35 °C with high humidity, culm elongation occurs; at low relative humidity, plants may be stunted (Naeem et al. 2016; Matic et al. 2017). Spores are airborne. *Fusarium* spores are among the most abundant in the aerial microbiota

during the rice growing season, even if no bakanae symptoms are evident in the field. It is difficult to breed rice varieties with a useful degree of resistance.

Since the 1970s, management of bakanae has been based on the use of protective fungicides. However, fungicide-resistant strains of the pathogen have appeared (Qiu et al. 2011; Ma et al. 2012; Chen et al. 2014).

Chemical, physical and biological seed dressing can be successful in controlling seedborne infection, but the best management strategies are based on the use of healthy seeds. Detection of infected seed lots is therefore important (Forsberg et al. 2003). Wulff et al. (2010) have reported infection rates of seed samples up to 9%. Some countries, such as Malaysia and Thailand, require imported rice seeds to be free of *F. fujikuroi*.

Molecular Tools to Resolve the Species Complex

sMolecular barcoding analysis has been applied to help resolve the GFSC species complex. Protein-coding genes providing the highest resolution include those for elongation factor, calmodulin (Carbone et al. 1999), ITS (O'Donnell and Cigelnik 1997), mtSSU (mitochondrial small subunit rDNA), beta tubulin, and 28S rDNA (O'Donnell et al. 1998). O'Donnell et al. (2000) determined that the elongation factor 1-alpha (EF1-α) and beta-tubulin were able to resolve 44 species within the GFSC. RNA polymerase II (RPB2) has also been used as a marker to distinguish species (Liu et al. 1999; Short et al. 2011; Choi et al. 2018). It has been concluded that EF1-α and RPB2 can successfully distinguish between species of GFSC (O'Donnell 1992; O'Donnell et al. 1998, 2000, 2007, 2009) using information from GenBank, the *Fusarium* database (Geiser et al. 2004), and genome sequencing projects (Jeong et al. 2013; Hwang et al. 2013). EF1-α has been used to identify the causal agent of bakanae disease in Italy (Amatulli et al. 2010).

PCR-based Assays

These characterization studies have provided a basis for the design of PCR-based assays that allow rapid identification of *F. fujikuroi* with specific primers. EF1-α has been used to design a qualitative PCR-based assay (Amatulli et al. 2012), and a TaqMan probe assay (Amaral Carneiro et al. 2017) that can be used to assay inoculum in rice culms, leaves, roots and seeds, with a limit of detection of approximately 10 fungal cells per gram of rice tissue. Sensitive targeted approaches such as TaqMan allow the fungus to be detected in asymptomatic tissue, thus accelerating decision-making in disease management. Other genetic regions have been used to design specific primers such as the unigene encoding *PNG1* (Hwang et al. 2013).

Such techniques are also useful to distinguish genotypes that present important virulence or fungicide resistance characteristics, for example the primer-introduced restriction analysis polymerase chain reaction (PIPA PCR) (Zhang et al. 2015).

LAMP assays for the detection of *F. fujikuroi* and *F. proliferatum* have been designed on the IGS (Intergenic Spacer region) and on the reductase-coding region (Rong et al. 2018). The LAMP products can be visualized by gel electrophoresis, by the precipitation of magnesium pyrophosphate, or by turbidimetric methods (Mori et al. 2004). The LAMP assay designed for *F. fujikuroi* and *F. proliferatum* is based on colour-changing reagents; for example, hydroxy naphthol blue (HNB) changes from violet to sky blue in the case of positive results (Goto et al. 2009). This colorimetric change can readily be detected in seeds and even in plants in the field, requiring only a bath for isothermal amplification. However, the colorimetric changes are not always consistent, so real-time methods based on fluorescence may be more suitable for in-field diagnosis. The use of portable equipment, such as the Genie II or Genie III instruments (OptiGene Ltd) allows for rapid detection of the LAMP products, with accurate results. Such devices can test for the presence of *F. fujikuroi* or other species on rice seeds, allowing certification of the absence of pathogens within a few minutes (Franco Ortega et al. 2018).

Conclusions

Accurate, effective, sensitive and specific diagnostic techniques are required for forecasting and management of rice diseases. Visual observation of symptoms in field crops, or of fungal growth on seed samples, is not always reliable. This chapter compares such methods with serological and especially molecular techniques that recognize the identity and measure the extent of fungal inoculum more precisely. PCR-based techniques have overtaken the use of culture-based methods in terms of speed, specificity and ease of use. The development of PCR techniques for use in the field is radically changing practical diagnostics, delivering precise results in a matter of hours rather than days or weeks.

For the future, there are exciting further possibilities of machine-learning and artificial intelligence (AI), perhaps based on concepts already outlined by Chung et al. (2016). AI systems may learn from experience of accumulated data, identify patterns and propose disease management recommendations with minimal human intervention. Coupling this with the specificity of genome-based pathogen characterization, it is reasonable to predict that some fresh approaches to plant disease control will be opened up, with promising implications for global food security – the subject of this book. Nevertheless, the dynamic of continuing plant pathogen evolution through natural selection will ensure that plant disease will remain as a continuing challenge.

Acknowledgements Some of the work reported here was carried out with funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634179 "Effective Management of Pests and Harmful Alien Species – Integrated Solutions" (EMPHASIS). The authors have no conflicts of interest to declare.

References

Ahn, I. P., Kim, S., Kang, S., Suh, S. C., & Lee, Y. H. (2005). Rice defense mechanisms against *Cochliobolus miyabeanus* and *Magnaporthe grisea* are distinct. *Phytopathology, 95*(11), 1248–1245. https://doi.org/10.1094/PHYTO-95-1248.

Almaguer, M., Rojas, T. I., & Hernández, A. (2008). Perspectivas de los estudios aeromicológicos para la protección del cultivo del arroz. *Revista de Protección Vegetal, 23*(3), 137–143.

Aluko, M. O. (1975). Crop losses caused by brown leaf spot disease of rice in Nigeria. *Plant Disease Report, 59*(7), 609–613.

Amaral Carneiro, G., Matic, S., Ortu, G., Garibaldi, A., Spadaro, D., & Gullino, M. L. (2017). Development and validation of a TaqMan real time PCR assay for the specific detection and quantification of *Fusarium fujikuroi* in rice plants and seeds. *Phytopathology, 107*(7), 885–892. https://doi.org/10.1094/PHYTO-10-16-0371-R.

Amatulli, M. T., Spadaro, D., Gullino, M. L., & Garibaldi, A. (2010). Molecular identification of *Fusarium* spp. associated with bakanae disease of rice in Italy and assessment of their pathogenicity. *Plant Pathology, 59*(5), 839–844. https://doi.org/10.1111/j.1365-3059.2010.02319.x.

Amatulli, M. T., Spadaro, D., Gullino, M. L., & Garibaldi, A. (2012). Conventional and real-time PCR for the identification of *Fusarium fujikuroi* and *Fusarium proliferatum* from diseased rice tissues and seeds. *European Journal of Plant Pathology, 134*(2), 401–408. https://doi.org/10.1007/s10658-012-9998-0.

Atkins, S. D., & Clark, I. M. (2004). Fungal molecular diagnostics: a mini review. *Journal of Applied Genetics, 45*(1), 3–15.

Ba, V. V., & Sangchote, S. (2006). Seedborne and transmission of *Bipolaris oryzae*, the causal pathogen of brown spot of rice. *Kasetsart Journal, Natural Sciences, 40*, 353–360.

Banerjee, S., Poswal, R., Gupta, S., Sharma, S., Bashyal, B. M., & Aggarwal, R. (2014). Molecular characterization of *Bipolaris* spp. using universal rice primer (URP) markers. *Indian Phytopathology, 67*(1), 49–54.

Barnwal MK, Kotasthane A, Magculia N, ;Mukherjee PK, Savary S, Sharma AK, Singh HB, Singh US, Spakrs AH, Variar M, Zaidi N (2013) A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. European Journal of Plant Pathology 136(3):443–457. doi: https://doi.org/10.1007/s10658-013-0195-6.

Barr, M. E. (1977). *Magnaporthe*, *Telimenella*, and *Hyponectria* (Physosporellaceae). *Mycologia, 69*(5), 952–966. https://doi.org/10.2307/3758778.

Berbee, M. L., Pirseyedi, M., & Hubbard, S. (1999). *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia, 91*(6), 964–977. https://doi.org/10.2307/3761627.

Bishaw, Z., Struik, P. C., & Van Gastel, A. J. G. (2012). Farmers' seed sources and seed quality: 1. Physical and physiological quality. *Journal of Crop Improvement, 26*(5), 655–692. https://doi.org/10.1080/15427528.2012.670695.

Biswas, S. K., Ratan, V., Srivastava, S. S. L., & Singh, R. (2008). Influence of seed treatment with biocides and foliar spraywith fungicides for management of brown leaf spot and sheath blight of paddy. *Indian Phytopathology, 61*(1), 55–59.

Borromeo, E. S., Nelson, R. J., Bonman, J. M., & Leung, H. (1993). Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. *The American Phytopathology Society, 83*(4), 393–399. https://doi.org/10.1094/Phyto-83-393.

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília: Mapa/ACS, 2009. 395p. online:http://www.agricultura.gov.br/arq_editor/file/12261_sementes_-web.pdf

Burgos, M. R. G., Katimbang, M. L. B., Dela Paz, M. A. G., Beligan, G. A., Goodwin, P. H., Mauleon, R. P., Ardales, E. Y., & Vera Cruz, C. M. (2013). Genotypic variability and aggressiveness of *Bipolaris oryzae* in the Philippines. *European Journal of Plant Pathology, 137*, 415–429. https://doi.org/10.1007/s10658-013-0256-x.

Carbone, I., Anderson, J. B., & Kohn, L. M. (1999). Patterns of descent in clonal lineages and their multilocus fingerprints are resolved with combined gene genealogies. *Evolution, 53*(1), 11–21. https://doi.org/10.2307/2640916.

Cartwright, R. D., Growth, D. E., Wamishe, Y. A., Greer, C. A., Calvert, L. A., Vera Cruz, C., Verdier, V., & Way, M. (2018). *Compendium of Rice diseases and pests* (2nd ed., p. 121). St Paul: APS press.

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. *Environmental Science & Policy, 9*(7–8), 685–692. https://doi.org/10.1016/j.envsci.2006.08.002.

Chakrabarti, N.K., Chaudhuri, S., Singh, U.S., Mukhopadhyay, A.N., Kumar, J. & Chaube, H.S. (1992). Brown spot of rice. Plant diseases of international importance. Volume I. *Diseases of cereals and pulses*. (pp. 116–129).

Chakrabarti, N. K. (2001). Epidemiology and disease management of brown spot of rice in India. In S. Screenivasaprasad & R. Johnson (Eds.), *Major fungal disease of Rice: Recent advances* (pp. 293–306). Dordrecht: Kluwer Academic Publishers.

Chadha, S., & Gopalakrishna, T. (2005). Retrotransposon-microsatellite amplified polymorphism (REMAP) markers for genetic diversity assessment of the rice blast pathogen (*Magnaporthe grisea*). *Genome, 48*(5), 943–945. https://doi.org/10.1139/G05-045.

Chadha, S., & Gopalakrishna, T. (2006). Detection of *Magnaporthe grisea* in infested rice seeds using polymerase chain reaction. *Journal of Applied Microbiology, 100*(5), 1147–1153. https://doi.org/10.1111/j.1365-2672.2006.02920.x.

Chen, C., Lian, B., Hu, J., Zhai, H., Wang, X., Venu, R. C., Liu, E., Wang, Z., Chen, M., Wang, B., Want, G. L., Wang, Z., & Mitchell, T. K. (2013). Genome comparison of two *Magnaporthe oryzae* field isolates reveals genome variations and potential virulence effectors. *BMC Genomics, 14*, 887. https://doi.org/10.1186/1471-2164-14-887.

Chen, Z., Gao, T., Liang, S., Liu, K., Zhou, M., & Chen, C. (2014). Molecular mechanism of resistance of *Fusarium fujikuroi* to benzimidazole fungicides. *FEMS Microbiology Letters, 357*(1), 77–84. https://doi.org/10.1111/1574-6968.12504.

Choi, J., Park, S. Y., Kim, B. R., Roh Jh, O. I. S., Han, S. S., & YHl, L. (2013). Comparative analysis of pathogenicity and phylogenetic relationship in *Magnaporthe grisea* species complex. *PLoS One, 8*(2), e57196. https://doi.org/10.1371/journal.pone.0057196.

Choi, J. H., Lee, S., Nah, J. Y., Kim, H. K., Paek, J. S., Lee, S., Ham, H., Hong, S. K., Yun, S. H., & Lee, T. (2018). Species composition of and fumonisin production by the *Fusarium fujikuroi* species complex isolated from Korean cereals. *International Journal of Food Microbiology, 267*, 62–69. https://doi.org/10.1016/j.ijfoodmicro.2017.12.006.

Chung, C. L., Huang, K. J., Chen, S. Y., Lai, M. H., Chen, Y. C., & Kuo, Y. F. (2016). Detecting Bakanae disease in rice seedlings by machine vision. *Computers and Electronics in Agriculture, 121*, 404–411. https://doi.org/10.1016/j.compag.2016.01.008.

Condon, B. J., Leng, Y., Wu, D., Bushley, K. R., Ohm, R. A., Otillar, R., Martin, J., Schachwitz, W., Grimwood, J., MohdZainudin, N., Xue, C., Wang, R., Manning, V. A., Dhillon, B., Jin Tu, Z., Steffenson, B. J., Salamov, A., Sun, H., Lowry, S., La Butti, K., Han, J., Copeland, A., Lindquist, E., Barry, K., Schmutz, J., Baker, S. E., Ciuffetti, L. M., Grigoriev, I. V., Zhong, S., & Turgeon, B. G. (2013). Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. *PLoS Genetics, 9*(1), e1003233. https://doi.org/10.1371/journal.pgen.1003233.

Couch, B. C., & Kohn, L. M. (2002). A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia, 94*, 683–693. https://doi.org/10.1080/15572536.2003.11833196.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J. R., Pan, H., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djovonic, S., Kolomiets, E., Rehmeyer, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bonhert, H., Couglan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E., & Birren, B. W. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature, 434*, 980–986. https://doi.org/10.1038/nature03449.

Dela Paz, M. A. G., Goodwin, P. H., Raymundo, A. K., Ardales, E. Y., & Vera Cruz, C. M. (2006). Phylogenetic analysis based on ITS sequences and conditions affecting the type of conidial germination of *Bipolaris oryzae*. *Plant Pathology, 55*(6), 756–765. https://doi.org/10.1111/j.1365-3059.2006.01439.x.

Delseny, M., Salses, J., Cooke, R., Sallaud, C., Regad, F., Lagoda, P., Guiderdoni, E., Ventelon, M., Brugidou, C., & Ghesquiere, A. (2001). Rice genomics: Present and future. *Plant Physiology and Biochemistry, 39*, 323–334. https://doi.org/10.1016/S0981-9428(01)01245-1.

Ellis, M. B., & Holliday, P. (1971). *Cochliobolus miyabeanus. Descriptions of fungi and bacteria* (p. 302). Wallingford: CAB International.

Eren, A. M., Maignien, L., Sul, W. J., Murphy, L. G., Grim, S. L., Morrison, H. G., & Sogin, M. L. (2013). Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene data. *Methods in Ecology and Evolution, 4*, 1111.

Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J. R., Pan, H., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomiets, E., Rehmeyer, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., & Galagan, J. E. (2005). The genome sequence of the rice blast fungus Magnaporthe *grisea*. *Nature, 434*, 980–986. https://doi.org/10.1038/nature03449.

Faivre-Rampant, O., Geniès, L., Piffanelli, P., & Tharreau, D. (2013). Transmission of rice blast from seeds to adult plants in a non-systemic way. *Plant Pathology, 62*(4), 879–887. https://doi.org/10.1111/ppa.12003.

Forsberg, G., Kristensen, L., Eibel, P., Titone, P., & Hart, W. (2003). Sensitivity of cereal seeds to short duration treatment with hot, humid air. *Journal of Plant Diseases and Protection, 110*(1), 1–16.

Franco Ortega, S., Tomlinson, J., Hodgetts, J., Spadaro, D., Gullino, M. L., & Boonham, N. (2018). Development of loop-mediated isothermal amplification assays for the detection of Seedborne fungal pathogens *Fusarium fujikuroi* and *Magnaporthe oryzae* in Rice seed. *Plant Disease, 102*(8), 1549–1558. https://doi.org/10.1094/PDIS-08-17-1307-RE.

Franco Ortega, S., Ferrocino, I., Adams, I., Silvestri, S., Spadaro, D., Gullino, M. L., & Boonham, N. (2020). Monitoring and surveillance of aerial mycobiota of rice paddy through DNA metabarcoding and qPCR. *Journal of Fungi, 6*(4), 372. https://doi.org/10.3390/jof6040372

Fujisaka, S., Guino, R. A., Lubigan, R. T., & Moody, K. (1993). Farmers' rice seed management practices and resulting weed seed contamination in the Philippines. *Seed Science and Technology, 21*(1), 149–157.

Gathala, M. K., Ladha, J. K., Kumar, V., Saharawat, Y., Kumar, V., Sharma Pradeep, K., Sharma, S., & Himanshu, P. (2011). Tillage and crop establishment affects sustainability of south Asian rice-wheat system. *Agronomy Journal, 103*(4), 961–971. https://doi.org/10.2134/agronj2010.0394.

Ge, S., Sang, T., Lu, B. R., & Hong, D. Y. (1999). Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proceedings of the National Academy of Sciences of the United States of America, 96*(25), 14400–14405. https://doi.org/10.1073/pnas.96.25.14400.

Geiser, D. M., Jiménez-Gasco, M. D. M., Kang, S., Makalowska, I., Narayanan, V., Ward, T. J., Zhang, N., Kuldau, A., & O'Donnell, K. (2004). FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology, 110*, 473–479. https://doi.org/10.1023/B:EJPP.0000032386.75915.a0.

George, M. L. C., Nelson, R. J., Zeigler, R. S., & Leung, H. (1998). Rapid population analysis of *Magnaporthe grisea* by using rep-PCR and endogenous repetitive DNA sequences. *Phytopathology, 88*(3), 223–229. https://doi.org/10.1094/PHYTO.1998.88.3.223.

Goto, M., Honda, E., Ogura, A., Nomoto, A., & Hanaki, K. (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *BioTechniques, 46*(3), 167–172. https://doi.org/10.2144/000113072.

Gowda, M., Shirke, M. D., & Mahesh, H. B. (2015). Genome analysis of rice-blast fungus *Magnaporthe oryzae* field isolates from southern India. *Genomics Data, 5*, 284–291. https://doi.org/10.1016/j.gdata.2015.06.018.

Gupta, A. K., Singh, Y., Jain, A. K., & Singh, D. (2014). Prevalence and incidence of bakanae disease of rice in northern India. *Journal of AgriSearch, 1*(4), 233–237.

Hamer, J. E., Farrall, L., Orbach, M. J., Valent, B., & Chumley, F. G. (1989). Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. *Genetics, 86*, 981–9985.

Hasegawa, H., Miyazawa, J., & Iwata, M. (1999). Use of ELISA to quantify *Magnaporthe grisea* in rice leaf. *Japanese Journal of Phytopathology, 65*(3), 312–314. https://doi.org/10.3186/jjphytopath.65.312.

Hebert, T. T. (1971). The perfect stage of *Pyricularia grisea*. *Phytopathology, 61*, 83–87. https://doi.org/10.1094/Phyto-61-83.

Hindson, B. J., Ness, K. D., Masquelier, D. A., Belgrader, P., Heredia, N. J., Makarewicz, A. J., Bright, I. J., Lucero, M. Y., Hiddessen, A. L., Legler, T. C., Kitano, T. K., Hodel, M. R., Petersen, J. F., Wyatt, P. W., Steenblock, E. R., Shah, P. H., Bousse, L. J., Troup, C. B., Mellen, J. C., Wittmann, D. K., Erndt, N. G., Cauley, T. H., Koehler, R. T., So, A. P., Dube, S., Rose, K. A., Montesclaros, L., Wang, S., Stumbo, D. P., Hodges, S. P., Romine, S., Milanovich, F. P., White, H. E., Regan, J. F., Karlin-Neumann, G. A., Hindson, C. M., Saxonov, S., & Colston, B. W. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry, 83*(22), 8604–8610. https://doi.org/10.1021/ac202028g.

Hindson, C. M., Chevillet, J. R., Briggs, H. A., Gallichotte, E. N., Ruf, I. K., Hindson, B. J., Vessella, R. L., & Tewari, M. (2013). Absolute quantification by droplet digital PCR versus analog real-time PCR. *Nature Methods, 10*(10), 1003–1005. https://doi.org/10.1038/nmeth.2633.

Hirst, J. M. (1954). An automatic volumetric spore trap. *The Annals of Applied Biology, 39*, 257–265.

Huang, C. M., Liao, D.-J., Wu, H.-S., Shen, W. C., & Chung, C. L. (2016). Cyclone-based spore trapping, quantitative real-time polymerase chain reaction and high resolution melting analysis for monitoring airborne inoculum of *Magnaporthe oryzae*. *The Annals of Applied Biology, 169*(1), 75–90.

Hwang, I. S., Kang, W.-R., Hwang, D. J., Bae, S. C., Yun, S. H., & Ahn, I. P. (2013). Evaluation of bakanae disease progression caused by Fusarium fujikuroi in Oryza sativa L. *Journal of Microbiology, 51*(6), 858–865. https://doi.org/10.1007/s12275-013-3472-3.

IRRI. (1988). International Rice research institute, Proc. symposium on sustainable agriculture: The role of green manure crops in rice farming systems. 25–29 may 1987.

ISTA, International Seed Testing Association. (2019). *International rules for seed testing*. https://www.seedtest.org/en/international-rules-_content%2D%2D-1%2D%2D1083.html

Ito, S., & Kimura, J. (1931). Studies on the 'bakanae' disease of the rice plant. *Reports Hokkaido National Agriculture Experiment Station, 27*, 1–95.

Jagadeesh, D., Prasanna Kumar, M. K., Chandrakanth, R., & Devaki, N. S. (2018). Molecular diversity of internal transcribed spacer among the monoconidial isolates of *Magnaporthe oryzae* isolated from rice in southern Karnataka, India. *Journal, Genetic Engineering & Biotechnology, 16*(2), 631–638. https://doi.org/10.1016/j.jgeb.2018.05.008.

Jeong, H., Lee, S., Choi, G. J., Lee, T., & Yun, S. H. (2013). Draft genome sequence of *Fusarium fujikuroi* B14, the causal agent of the Bakanae disease of Rice. *Genome Announcements, 1*(1), e00035-13–e00035-13. https://doi.org/10.1128/genomeA.00035-13.

Johnson, D. R., & Percich, J. A. (1992). Wildrice domestication, fungal brown spot disease and the future of commercial production in Minnesota. *Plant Disease, 76*, 1193–1198. https://doi.org/10.1094/PD-76-1193.

Joshi, R. C., Matchoc, O. R. O., Bahatan, R. G., & Dela, P. F. A. (2000). Farmers' knowledge, attitudes and practices of rice crop and pest management at Ifugao Rice terraces, Philippines. *International Journal of Pest Management, 46*(1), 43–48. https://doi.org/10.1080/096708700227561.

Kato, H., Yamamoto, M., Yamaguchi-Ozaki, T., Kadouchi, H., Iwamoto, Y., Nakayashiki, H., Tosa, Y., Mayama, S., & Mori, N. (2000). Pathogenicity, mating ability and DNA restriction fragment length polymorphisms of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. *Journal of General Plant Pathology, 66*(1), 30–47. https://doi.org/10.1007/PL00012919.

Kohls, C. L., Percich, J. L., & Huot, C. M. (1987). Wild rice yield losses associated with growth stages specific fungal brown spot epidemics. *Plant Disease, 71*(5), 419–422. https://doi.org/10.1094/PD-71-0419.

Khush, G. S. (2005). What it will take to feed 5.0 billion Rice consumers in 2030. *Plant Molecular Biology, 59*(1), 1–6. https://doi.org/10.1007/s11103-005-2159-5.

Khush, G. S., & Jena, K. K. (2009). Current status and future prospects for research on blast resistance in Rice (*Oryza sativa* L.). In G. L. Wang & B. Valent (Eds.), *Advances in genetics, genomics and control of rice blast disease* (pp. 1–10). Dordrecht: Springer Netherlands.

Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., de Vries, R. P., & Lebrun, M. H. (2014). Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae). *Studies in Mycology, 79*, 85–120. https://doi.org/10.1016/j.simyco.2014.09.004.

Koizumi, S., Ashizawa, T., & Zenbayashi, K. S. (2004). Durable control of Rice blast disease with multilines. In S. Kawasaki (Ed.), *Rice blast: Interaction with Rice and control* (pp. 191–199). Dordrecht: Springer Netherlands.

Kuribayashi, K. (1928). Studies on everwinteringprimary infection and control of rice blast fungus, *Pyricularia oryzae*. *Annals of the Phytopathological Society of Japan, 2*, 99–117.

Lee, F.N., Scott, H.A., & Xia, J.Q. (1998). *Serological detection and identification of rice blast*. US Patent NO, 5,789,183.

Leung, H., Borromeo, E. S., Bernardo, M. A., & Notteghem, J. L. (1988). Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. *Phytopathology, 78*, 1227–1233. https://doi.org/10.1094/Phyto-78-1227.

Liu, Y. J., Whelen, S., & Hall, B. D. (1999). Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution, 16*(12), 1799–1808. https://doi.org/10.1086/523946.

Luo, Y., Ma, Z., Reyes, H. C., Morgan, D., & Michailides, T. J. (2007). Quantification of airborne spores of *Monilinia fructicola* in stone fruit orchards of California using real-time PCR. *European Journal of Plant Pathology, 118*(2), 145–154. https://doi.org/10.1007/s10658-007-9124-x.

Ma, X., Xing, C., Yu, J., Wang, Y., Chen, Z., Wang, J., Zhou, M., & Chen, C. (2012). Cloning beta-tubulin from *Fusarium fujikuroi* to analyze its relationship with carbendazim resistance. *Wei Sheng Wu Xue Bao, 52*(2), 581–587. (In Chinese).

Manamgoda, D. S., Cai, L., McKenzie, E. H. C., Crous, P. W., MAdrid, H., Chukeatirote, E., Shivas, R. G., Tan, Y. P., & Hyde, K. D. (2012). A phylogenetic and taxonomic re-evaluation of the *Bipolaris – Cochliobolus – Curvularia* complex. *Fungal Diversity, 56*(1), 131–144. https://doi.org/10.1007/s13225-012-0189-2.

Manandhar, H. K., & Jorgensen, H. (1998). Seedborne infection of rice by *Pyricularia oryzae* and its transmission to seedlings. *Plant Disease, 82*(10), 1093–1099. https://doi.org/10.1094/PDIS.1998.82.10.1093.

Mathur, S. B. (1995). Some aspects of seed pathology that deserve immediate attention. *Indian Journal of Mycology and Plant Pathology, 25*, 13–24.

Matic, S., Gullino, M. L., & Spadaro, D. (2017). The puzzle of bakanae disease through interactions between *Fusarium fujikuroi* and rice. *Frontiers in Bioscience, 9*, 333–344. https://doi.org/10.2741/e806.

Meitz-Hopkins, J. C., von Diest, S. G., Koopman, T. A., Bahramisharif, A., & Lennox, C. L. (2014). A method to monitor airborne *Venturia inaequalis* ascospores using volumetric spore traps and quantitative PCR. *European Journal of Plant Pathology, 140*(3), 527–541. https://doi.org/10.1007/s10658-014-0486-6.

Mew, T. W., & Gonzales, P. (2002). *A handbook of Rice Seedborne Fungi*. New Hampshire: International Rice Research Institute. Science Publishers.

Mohd Zainudin, N. A. I., Razak, A. A., & Salleh, B. (2008). Bakanae disease of rice in Malaysia and Indonesia: Etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. *Journal of Plant Protection Research, 48*(4), 475–485. https://doi.org/10.2478/v10045-008-0056-z.

Mori, Y., Kitao, M., Tomita, N., & Notomi, T. (2004). Real-time turbidimetry of LAMP reaction for quantifying template DNA. *Journal of Biochemical and Biophysical Methods, 59*(2), 145–157. https://doi.org/10.1016/j.jbbm.2003.12.005.

Motallebi, P., Javan-Nikkhah, M., Okhovvat, M., Berdi Fotouhifar, K., & Hossien Mosahebi, G. (2009). Differentiation of *Magnaporthe* species complex by rep-PCR genomic fingerprinting. *Communications in Agricultural and Applied Biological Sciences, 74*(3), 821–829.

Moura, A. B., Ludwig, J., Garske Santos, A., Tavares Schafer, J., Nogueira Soares, V., & Corrêa, O. (2014). Biocontrol and seed transmission of *Bipolaris oryzae* and *Gerlachia oryzae* to rice seedlings. *Journal of Seed Science, 36*(4), 407–412. https://doi.org/10.1590/2317-1545v36n41009.

Naeem, M., Iqbal, M., Parveen, N., & Ul-Allh, S. (2016). An over view of Bakanae disease of Rice. *American-Eurasian Journal of Agricultural & Environmental Sciences, 16*(2), 270–277. https://doi.org/10.5829/idosi.aejaes.2016.16.2.12834.

Nagamine, K., Hase, T., & Notomi, T. (2002). Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Molecular and Cellular Probes, 16*(3), 223–229.

Nakayashiki, H., Nishimoto, N., Ikeda, K., Tosa, Y., & Mayama, S. (1999). Degenerate MAGGY elements in a subgroup of *Pyricularia grisea*: A possible example of successful capture of a genetic invader by a fungal genome. *Molecular Genetics and Genomics, 261*(6), 958–966.

Nayar, N. M. (2014). Chapter 3 – phylogeny of the genus *Oryza* L. In N. M. Nayar (Ed.), *Origin and phylogeny of Rices* (pp. 37–57). San Diego: Academic.

Neergaard, P., Lambat, A. K., & Mathur, S. B. (1970). Seed health testing of rice III: Testing procedure for detection of *Pyricularia oryzae* Cav. *Proceedings of the International Seed Testing Association, 35*, 157–163.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research, 28*(12), E63. https://doi.org/10.1093/nar/28.12.e63.

Nyvall, R. F. (1995). Comparison of fungal Brown spot severity to incidence of Seedborne *Bipolaris oryzae* and *B. sorokiniana* and infected floral sites on cultivated wild Rice. *Plant Disease, 79*(3), 249–250. https://doi.org/10.1094/PD-79-0249.

O'Donnell, K. (1992). Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics, 22*(3), 213–220. https://doi.org/10.1007/BF00351728.

O'Donnell, K., & Cigelnik, E. (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution, 7*(1), 103–116. https://doi.org/10.1006/mpev.1996.0376.

O'Donnell, K., Cigelnik, E., & Nirenberg, H. I. (1998). Molecular systematics and Phylogeography of the Gibberella fujikuroi species complex. *Mycologia, 90*(3), 465–493. https://doi.org/10.2307/3761407.

O'Donnell, K., Nirenberg, H., Aoki, T., & Cigelnik, E. (2000). A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience, 41*(1), 61–78. https://doi.org/10.1007/bf02464387.

O'Donnell, K., Sarver, B. A. J., Brandt, M., Chang, D. C., Noble-Wang, J., Park, B. J., Sutton, D. A., Benjamin, L., Lindsley, M., Padhye, A., Geiser, D. M., & Ward, T. J. (2007). Phylogenetic diversity and microsphere array-based genotyping of human pathogenic *Fusaria*, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology, 45*(7), 2235–2248. https://doi.org/10.1128/JCM.00533-07.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Gueidan, C., Crous, P. W., & Geiser, D. M. (2009). Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and F. *chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology, 47*, 3851–3861. https://doi.org/10.1128/JCM.01616-09.

Ohata, K. (1989). Brown spot disease. (in Japanese). In Z. Nouson & K. Kyoukai (Eds.), *Rice diseases* (pp. 357–374). Tokyo: Zenkoku Nouson Kyouiku Kyoukai.

Olivier, C., Berbee, M. L., Shoemaker, R. A., & Loria, R. (2000). Molecular phylogenetic support from ribosomal DNA sequences for origin of *Helminthosporium* from *Leptosphaeria*-like Loculoascomycete ancestors. *Mycologia, 92*(4), 736–746. https://doi.org/10.2307/3761430.

Ou, S. H. (1985). *Rice diseases* (2nd ed., p. 380). Kew: The Commonwealth Mycological Institute.

Padmanabhan, S. Y., & Ganguly, D. (1954). Relation between the age of rice plant and its susceptibility to *Helminthosporium* and blast diseases. *Proceedings of the Indian Academy of Sciences – Section B, 39*(2), 44–50. https://doi.org/10.1007/BF03050372.

Padmanabhan, S. Y. (1973). The great Bengal famine. *Annual Review of Phytopathology, 11*, 11–26.

Padmanabhan, S.Y. (1977). Fungal Diseases of Rice in India. Indian Council of Agricultural Research. New Delhi, India pp 66.

Percich, J. A., Nyvall, R. F., Malvick, D. K., & Kohls, C. L. (1997). Interaction of temperature and moisture on infection of wild rice by *Bipolaris oryzae* in the growth chamber. *Plant Disease, 81*(10), 1193–1195. https://doi.org/10.1094/PDIS.1997.81.10.1193.

Piombo, E., Abdelfattah, A., Droby, S., Wisniewski, M., Spadaro, D., & Schena, L. (2021). Metagenomics approaches for the detection and surveillance of emerging and recurrent plant pathogens. *Microorganisms, 9*(1), 188. https://doi.org/10.3390/microorganisms9010188.

Qiu, J., Xu, J., Yu, J., Bi, C., Chen, C., & Zhou, M. (2011). Localisation of the benzimidazole fungicide binding site of Gibberella zeae β2-tubulin studied by site-directed mutagenesis. *Pest Management Science, 67*(2), 191–198. https://doi.org/10.1002/ps.2050.

Rong, Z., Yuan, Y., Ye, W., Wang, X., & Zheng, X. (2018). Rapid diagnosis of rice bakanae caused by *Fusarium fujikuroi* and *F. proliferatum* using loop-mediated isothermal amplification assays. *Journal of Phytopathology, 66*(4), 283–290. https://doi.org/10.1111/jph.12685.

Sato, H., Ando, I., Hirabayashi, H., Hirabayashi, H., Takeuchi, Y., Arase, S., Kihara, J., Kato, H., Imbe, T., & Nemoto, H. (2008). QTL analysis of brown spot resistance in rice (*Oryza sativa* L.). *Breeding Science, 58*, 93–96.

Savary, S., Castilla, N. P., Elazegui, F. A., & Teng, P. S. (2005). Multiple effects of two drivers of agricultural change, labour shortage and water scarcity, on rice pest profiles in tropical Asia. *Field Crops Research, 91*(2–3), 263–271. https://doi.org/10.1016/j.fcr.2004.07.017.

Savary, S., Mila, A., Willocquet, L., Esker, P. D., Carisse, O., & McRoberts, N. (2011a). Risk factors for crop health under global change and agricultural shifts: A framework of analyses using rice in tropical and subtropical Asia as a model. *Phytopathology, 101*(6), 696–709. https://doi.org/10.1094/PHYTO-07-10-0183.

Savary, S., Nelson, A., Sparks, A. H., & Djurle, A. (2011b). International agricultural research tackling the effects of global and climate changes on plant diseases in the developing world. *Plant Disease, 95*(10), 1204–1216. https://doi.org/10.1094/PDIS-04-11-0316.

Savary, S., Nelson, A., Willocquet, L., Pangga, I., & Aunario, J. (2012). Modeling and mapping potential epidemics of rice diseases globally. *Crop Protection, 34*, 6–17. https://doi.org/10.1016/j.cropro.2011.11.009.

Savary, S., Teng, P. S., Willocquet, L., & Nutter, F. W. (2006). Quantification and modeling of crop losses: A review of purposes. *Annual Review of Phytopathology, 44*, 89–112. https://doi.org/10.1146/annurev.phyto.44.070505.143342.

Savary, S., Willocquet, L., & Teng, P. S. (1997). Modelling sheath blight epidemics on rice tillers. *Agricultural Systems, 55*(3), 359–384. https://doi.org/10.1016/S0308-521X(97)00014-0.

Sawada, K. (1919). Descriptive catalogue of the Formosan fungi. *Rept Department of Agriculture, Government Research Institute, Formosa, 19*, 1–695.

Schaad, N. W., Frederick, R. D., Shaw, J., Schneider, W. L., Hickson, R., Petrillo, M. D., & Luster, D. G. (2003). Advances in molecular-based diagnostics in meeting crop biosecurity and phytosanitary issues. *Annual Review of Phytopathology, 41*, 305–324. https://doi.org/10.1146/annurev.phyto.41.052002.095435.

Sharma, V. K., & Thind, T. S. (2007). Rice diseases: Ecology and control. In D. Pimentel (Ed.), *Encyclopedia of Pest management* (CRC press) (Vol. II, pp. 556–561). Abingdon: Taylor and Francis Group.

Short, D. P. G., O'Donnell, K., Zhang, N., Juba, J. H., & Geiser, D. M. (2011). Widespread occurrence of diverse human pathogenic types of the fungus *Fusarium* detected in plumbing drains. *Journal of Clinical Microbiology, 49*(12), 4264–4272. https://doi.org/10.1128/JCM.05468-11.

Siciliano, I., Amaral Carneiro, G., Spadaro, D., Garibaldi, A., & Gullino, M. L. (2015). Jasmonic acid, abscisic acid, and salicylic acid are involved in the phytoalexin responses of rice to *Fusarium fujikuroi*, a high gibberellin producer pathogen. *Journal of Agricultural and Food Chemistry, 63*(37), 813–842. https://doi.org/10.1021/acs.jafc.5b03018.

Skamnioti, P., & Gurr, S. J. (2009). Against the grain: Safeguarding rice from rice blast disease. *Trends in Biotechnology, 27*(3), 141–150. https://doi.org/10.1016/j.tibtech.2008.12.002.

Sone, T., Fukiya, S., Kodama, M., & Tomita, F. (2000). Molecular structure of rDNA repeat unit in *Magnaporthe grisea*. *Bioscience, Biotechnology, and Biochemistry, 64*(8), 1733–1736.

Sprague, R. (1950). *Diseases of cereals and grasses in North America* (p. 538). New York: Ronald Press.

Sun, S. K., & Snyder, W. C. (1981). The bakanae disease of the rice plant. In P. E. Nelson, T. A. Toussoun, & R. J. Cook (Eds.), *Fusarium: Diseases, biology and taxonomy* (pp. 104–113). Pennsylvania: The Pennsylvania State University Press, University Park.

Su'udi, M., Kim, J., Park, J. M., Bae, S. C., Kim, D., Kim, Y. H., & Ahn, I. P. (2013). Quantification of rice blast disease progressions through Taqman real-time PCR. *Molecular Biotechnology, 55*(1), 43–48. https://doi.org/10.1007/s12033-012-9632-6.

Su'udi, M., Park, J. M., Kang, W. R., Park, S. R., Hwang, D. J., & Ahn, I. P. (2012). Quantification of rice brown leaf spot through Taqman real-time PCR specific to the unigene encoding *Cochliobolus miyabeanus* Scytalone Dehydratase1 involved in fungal melanin biosynthesis. *Journal of Microbiology, 50*(6), 947–954. https://doi.org/10.1007/s12275-012-2538-y.

Talbot, N. J. (2003). On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology, 57*, 177–202. https://doi.org/10.1146/annurev.micro.57.030502.090957.

Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H., & Kushiro, M. (2007). Mycotoxins in rice. *International Journal of Food Microbiology, 119*(1–2), 59–66. https://doi.org/10.1016/j.ijfoodmicro.2007.08.002.

Taylor, S. C., Laperriere, G., & Germain, H. (2017). Droplet digital PCR versus qPCR for gene expression analysis with low abundant targets: From variable nonsense to publication quality data. *Scientific Reports UK, 7*, 2409.

Tomlinson, J. A., Boonham, N., & Dickinson, M. (2010). Development and evaluation of a one-hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas. *Plant Pathology, 59*(3), 465–471. https://doi.org/10.1111/j.1365-3059.2009.02233.x.

Tosa, Y., & Chuma, I. (2014). Classification and parasitic specialization of blast fungi. *Journal of General Plant Pathology, 80*(3), 202–209.

United Nations, Department of Economic and Social Affairs, Population Division. (2015). *World population prospects: The 2015 revision, key findings and advance tables*. Working Paper No. ESA/P/WP.241.

Vidhyasekharan, P., Ramadoss, N., & Srinivasalu, N. (1973). Quantitative and qualitative losses in paddy due to Helminthosporiose epidemic. *Indian Phytopathology, 26*, 479–484.

Villari, C., Mahaffee, W. F., Mitchell, T. K., Pedley, K. F., Pieck, M. L., & Peduto Hand, F. (2017). Early detection of airborne inoculum of *Magnaporthe oryzae* in turfgrass fields using a quantitative LAMP assay. *Plant Disease, 101*, 170–177. https://doi.org/10.1094/PDIS-06-16-0834-RE.

Ward, E., Tahri-Alaoui, A., & Antoniw, J. E. (1998). Applications of PCR in plant-fungal interaction. In P. D. Bridges, D. K. Arroar, C. A. Reddy, & R. P. Elander (Eds.), *Application of PCR in mycology* (pp. 289–307). London: CAB international.

Wulff, E. G., Sørensen, J. L., Lübeck, M., Nielsen, K. F., Thrane, U., & Torp, J. (2010). *Fusarium* spp. associated with rice Bakanae: Ecology, genetic diversity, pathogenicity and toxigenicity. *Environmental Microbiology, 12*(3), 649–657. https://doi.org/10.1111/j.1462-2920.2009.02105.x.

Xia, J. Q., Lee, F. N., Scott, H. A., & Raymond, L. R. (1992). Development of monoclonal antibodies specific for Pyricularia grisea, the rice blast pathogen. *Mycological Research, 96*(10), 867–873. https://doi.org/10.1016/S0953-7562(09)81033-2

Xue, M., Yang, J., Li, Z., Hu, S., Yao, N., Dean, R. A., Zhao, W., Shen, M., Zhang, H., Li, C., Liu, L., Cao, L., Xu, X., Xing, Y., Hsiang, T., Zhang, Z., Xu, J. R., & Peng, Y. L. (2012). Comparative analysis of the genomes of two field isolates of the Rice blast fungus *Magnaporthe oryzae*. *PLoS Genetics, 8*(8), e1002869. https://doi.org/10.1371/journal.pgen.1002869.

Zanão Júnior, L. A., Rodrigues, F. Á., Fontes, R. L. F., Korndörfer, G. H., & Lima Neves, J. C. (2009). Rice resistance to Brown spot mediated by silicon and its interaction with manganese. *Journal of Phytopathology, 157*(2), 73–78. https://doi.org/10.1111/j.1439-0434.2008.01447.x.

Zhang, Z., Chen, Z., Hou, Y., Duan, Y., Wang, J., Zhour, M., & Chen, C. (2015). PIRA PCR for discriminate genotypes with fungicide resistance alleles. *Plant Disease, 99*, 1241–1246.

Chapter 10
Automated Detection of '*Ca*. Liberibacter asiaticus' Infection in Citrus Using Immune Tissue Prints and Machine Learning

Jonathan Shao, Fang Ding, Shimin Fu, and John Hartung

Abstract Huanglongbing (HLB) or citrus greening disease has caused unprecedented losses to the citrus industry in Florida since its discovery there in 2005. HLB is caused by an unculturable member of the alpha-proteobacteria '*Ca*. Liberibacter asiaticus' (Clas). Diagnosis of HLB is by identification of the pathogen in infected trees, which are then often destroyed to prevent spread of the disease. Identification of the pathogen is generally by PCR-based methods that are expensive, labor-intensive and require isolation of DNA from tree samples; furthermore, non-uniform distribution of the pathogen in each tree causes a high rate of 'false negative' results. We have previously developed an immune tissue print assay to supplement PCR-based testing. The assay scales well to large numbers of samples and produces digital images of cross sections of plant tissue that have been serologically probed to reveal the presence of Clas. The assay relies on a human expert to classify the image as infected or not infected – a process that can be tedious and subject to inconsistency and bias. To address this, we trained a convolutional neural network (CNN) based on Inception V3 architecture available in Tensorflow to interpret the images. Sets of curated images were prepared from healthy or diseased petioles or stems tested by the immune tissue print assay, and were used to train the CNN models.

J. Shao
United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA
e-mail: jonathan.shao@usda.gov

F. Ding
Huazhong Agricultural University, Wuhan, Hubei, People's Republic of China

S. Fu
Southwest University, Chongqing, People's Republic of China

J. Hartung (✉)
United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA
e-mail: john.s.hartung@gmail.com

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_10

Curated sets of positive and negative training images were combined and submitted as unknowns to the CNN models for interpretation. The petiole-based model classified 81% of images in agreement with the curator at more than 90% confidence – slightly more than the stem model. Similar results were obtained when images that were not used to train the models were submitted as unknowns. Two instances were noted in which the model incorrectly classified a plainly infected image. This application of machine learning, with appropriate curator oversight, has the potential to deliver reliable interpretation of tissue print assay results, for the security of citrus production. It may also be useful in diagnosis of other diseases of citrus in which the pathogen has a phloem-limited distribution.

Introduction

Huanglongbing (HLB) or citrus greening disease (Bové 2006; Wang and Trivedi 2013) has caused unprecedented losses to the citrus industry in Florida since its discovery there in 2005. Sweet orange and grapefruit production has been reduced by 80 and 96% respectively. HLB is also present in Texas and California and indeed has become a global problem for citriculture since its origin in Fujian, China (Bové 2006) and first description in India (Nath and Husain 1927). Sustained and productive research has greatly increased the understanding of HLB since its discovery in Florida in 2005, but control measures remain insufficient.

Control of the disease remains elusive because the disease presents a constellation of characteristics that make it extraordinarily difficult to make modifications to the production system to achieve control (Gottwald and McCollum 2017). In spite of several reports that could not be verified, the bacterium associated with HLB, '*Ca*. Liberibacter asiaticus' (CLas) cannot be grown in the laboratory, which prevents the tools of bacterial genetics from being applied to the problem. CLas also multiplies in the citrus psyllid, *Diaphorina citri* (Kuwayama) and is spread among trees by this insect vector. The vector is small and prolific and is itself difficult to detect at early stages of infestation. The psyllids prefer to feed on and lay eggs in very tender growth of young leaves (the flush). Citrus trees put out new flush in response to environmental conditions, and, in Florida, citrus can put out flush four times per year, meaning there is nearly always susceptible tissue present for both the psyllid and the pathogen. All varieties of commercial citrus are susceptible to HLB, and because citrus is produced as a clonally propagated tree fruit, possibilities for breeding for resistance in the host are limited, although progress has been made (Deng et al. 2019; Grosser and Gmitter 2016). Another factor in the epidemiology of HLB is the encroachment of the urban and suburban environment on the citrus industry. The citrus psyllid, CLas and HLB persist in private homeowner properties regardless of control measures by the citrus industry. Indeed, HLB was first discovered in Miami, Florida (Knighten et al. 2005) and adjacent urbanized communities in south Florida, and in the southern California metroplex (Lyle 2012, 2015). Further

extension of human populations onto agricultural land – and increased international trade and travel that allows uncontrolled movement of pathogens and pests – present challenges for food security that can be expected to accelerate in the twenty-first century.

CLas infections begin when infected psyllids feed on emerging leaf flush (Bové 2006) but the bacterium moves rapidly to the roots, and from there becomes systemic in trees. Symptoms do not become apparent until after the root system has been seriously damaged (Graham et al. 2013). There is a prolonged asymptomatic stage of disease progression in which symptoms are not observed but the psyllid can acquire the pathogen and transmit to neighboring trees. This cryptic phase of infection explains why HLB was found throughout the Florida citrus industry within months of its initial discovery: latent infections established earlier progressed to the symptomatic stage simultaneously (Gottwald et al. 2007). The prolonged latent periods that allow spread of the disease before control measures are implemented makes accurate and specific diagnosis of CLas infection before the manifestation of HLB disease a high priority for research.

Detection of CLas for diagnosis of HLB is generally done today by qPCR-based methods using symptomatic leaf tissue to enable simple sampling protocols (Li et al. 2006; Morgan et al. 2012; Zheng et al. 2016). Although these methods are very sensitive, specific, well validated and accepted (Anonymous 2014) they are useful only to confirm a preliminary diagnosis based on HLB disease symptoms. This is because although CLas is distributed systemically in diseased trees, its distribution is not at all uniform, but is instead quite sporadic (Hartung et al. 2010; Li et al. 2009; Tatineni et al. 2008), and it is very unlikely that any asymptomatic tissue selected for testing will contain the pathogen. This complicates any sampling regimen devised for plant testing, because CLas may not be detected in some samples from plainly symptomatic trees, and a single citrus tree has a great many leaves. CLas lives in plants exclusively in the phloem tissues, which can be thought of as a system of tubes used by the plant to move sugar between tissues. Because the phloem vessels are woody, extraction of DNA prior to PCR-based assays is cumbersome, expensive and time consuming, and leads to poor yield of CLas DNA. The expense and labor required to carry out large-scale sampling of trees, either on an in-depth basis for a single tree or of many trees over a commercial grove, severely limits the utility of qPCR for this purpose.

To provide an alternative detection paradigm to qPCR, we have developed an immune tissue print assay (Cassab and Varner 1987) for CLas. To do this, we used genome sequence data for CLas (Duan et al. 2009) to predict the amino acid sequence of proteins on the surface of CLas, and used PCR-based cloning to express and purify them from *E. coli* (Liu et al. 2017), to immunize rabbits (Ding et al. 2016). The antibodies were used in a tissue-print format to visually detect CLas in infected citrus as purple spots in the phloem cells of infected citrus (Ding et al. 2015; Ding et al. 2016). Low-power magnification produced digital images that were stored for later interpretation and analysis. A bottleneck in the process of using tissue prints is that an expert must visually inspect each image and make a judgement as to whether it confirms the presence of CLas in the sample. This is usually

straightforward, and provides what may be a misleadingly qualitative yes/no decision. The analysis also becomes tedious.

To address these problems, we were interested in an approach to image analysis based on machine learning. Traditional neural networks are based on a multilayer perceptron (MLP). The MLP approach has prohibitive computational costs, and is translationally variant, meaning the location of a small relevant image within a larger image is critically important for correct classification of the image (Stewart 2019). This would constrain the acquisition of the tissue-print image by imposing a potentially severe penalty for off-center images, which would complicate image acquisition. For these reasons, we have begun to develop and implement a supervised representation learning-based approach to train convolutional neural networks (CNNs) (Stewart 2019) to evaluate the images and provide confidence scores associated with the determination of a given image as either positive or negative for the presence of CLas. By using this approach the acquisition and submission of the images to the CNN is simplified, and the reliability of the image classification produced by the CNN itself is improved.

Materials and Methods

Some of the images used to create and test this model were prepared for previous studies (Ding et al. 2015, 2016; Fu et al. 2019). Other images were from tissue prints prepared at the United States Repository for Citrus and Dates at the University of California, Riverside. The tissue prints were prepared by cutting stems or petioles from greenhouse-grown (Beltsville) or field-grown (Riverside) trees and pressing the freshly cut ends of the stems or petioles onto nitrocellulose paper (Thermo Scientific #88018). At Riverside, 80 tissue prints were prepared on each piece of nitrocellulose in 8 × 10 arrays and sent to Beltsville for serological detection. Tissue prints from known CLas-positive and CLas-negative trees were added in Beltsville to each 8 × 10 array as positive and negative controls.

Serological detection used a rabbit polyclonal antibody raised against a fragment of an outer membrane protein of CLas (YP_003065185.1) (Liu et al. 2017). Detection of the rabbit antibody bound to the surface of the tissue prints was by incubation with a goat anti-rabbit antibody conjugated with alkaline phosphatase (EMD-Millipore #12–448) followed by the addition of the substrate NBT and BCIP (Sigma Aldrich, St. Louis, MO). Incubation was continued until purple color could be seen. Tissue prints were photographed with a stereo Discovery V20 light microscope (Carl Zeiss, Jena, Germany) equipped with an AxioCamHR3 digital camera (Ding et al. 2016).

The images from petioles and stems were processed separately, and training and test sets were created for each tissue type. The training set for "Model-3" images of stems or petioles consisted of 122 or 130 images respectively. For each training set, the images were curated and sorted into bins, either positive or negative for Clas (Table 10.1). Each image was rotated through 90°, 180°, 270° and 360° angles using

Table 10.1 Assessment of prediction models when the images of tissue prints used as training sets for stem and petiole models were submitted to the models as unknown. Images were classified by the curator as known negative or known positive

Stem				Petiole			
Known negative		Known positive		Known negative		Known positive	
Model 3							
Confidence >0.9				Confidence >0.9			
33/49	67%	47/73	64%	65/88	74%	30/42	71%
Confidence >0.80				Confidence >0.80			
40/49	82%	58/73	80%	78/88	89%	40/42	95%
Model 4							
Confidence >0.9				Confidence >0.9			
32/48	67%	47/73	64%	64/78	82%	30/37	81%
Confidence >0.80				Confidence >0.80			
39/48	81%	58/73	80%	69/78	89%	35/37	95%

the software program ImageMagic-7.0.8-25 as a means of data augmentation to increase the number of samples available to train the models. The images were converted to jpeg format and spaces in image file names were changed to underscores, to standardize image format and naming. In "Model 4" the training images were curated a second time with more stringent standards for declaring an image to be positive or negative. Images that were ambiguous or from tissue prints that were poorly prepared were removed (Table 10.1).

The models were validated by submitting the training images as unknowns, and asking the model to estimate the probability that each image was positive or negative for the presence of CLas. These probabilities along with image identification tags were used to create a spreadsheet. Then the curator evaluated each image again and declared it to be positive or negative for the presence of CLas without reference to the probabilities already assigned by the model. The results of the human curator and the model were then compared. The determinations of the human and machine experts were compared by creating 2×2 contingency tables using various probability thresholds to declare the machine expert probabilities to be positive or negative for the presence of CLas.

Larger sets of images that included images not used to train the model were then submitted to the validated models, and the models were asked to test the null hypothesis that the image contained evidence for infection by CLas.

In supervised machine learning, such as we carried out in this work, the algorithm or convolutional neural network is trained on curated and labeled datasets. The training sets with labels for images of petioles (petiole_positive, petiole_negative) and the training sets with labels for images of stems (stem_positive, stem_negative) were retrained using the retrain.py script using bottleneck with 1000 training steps. In a convolutional neural network such as we used there are several computational layers. The bottleneck layer is the layer that occurs just before the final output layer that performs the classification step. The convolutional neural network (CNN)

trained model (https://github.com/tensorflow/hub/raw/master/examples/image_retraining/retrain.py) used the Inception V3 architecture, inception-2015-12-05.tgz (https://www.tensorflow.org/). The result is a retrained graph that can be used for discriminating between tissue prints positive and negative for CLas. During the retraining process, a series of three calculations are performed, the validation accuracy (separate training set), training accuracy (test data), and cross entropy, which is a loss function that gives the status of the learning process. During the training process, the tensorflow workflow sequesters some of the inputted training data for validation in order to validate the model (https://www.tensorflow.org/hub/tutorials/image_retraining). Using a python script, the test data (images) are read in, the labels are loaded and the images are input into the retrained graph and confidence scores are produced and sorted. This analysis was carried out on the USDA-ARS computational cluster Ceres on ARS SCINet, a high-performance computing environment with 2556 CPU cores in 96 computer nodes with 30 TB of memory. This experiment was performed on one of these nodes, an Intel® Xeon 2.30 GHz with 375 GB of RAM and 72 processing units.

Results

Tissue prints of both stem and petiole sections were prepared on nitrocellulose membranes and incubated with the primary rabbit antibody raised against the outer membrane epitope and the secondary goat anti-rabbit antibody conjugated with alkaline phosphatase with substrate. The anatomy of the stem was preserved in the tissue prints, with the principal tissue types plainly visible (Fig. 10.1a). Microcolonies of CLas could be readily seen as discrete purple spots filling individual phloem cells of stem sections taken from CLas-infected trees. In consecutive serial sections from the same stem section the pattern of infected phloem cells was correlated between the sections (Fig. 10.1a).

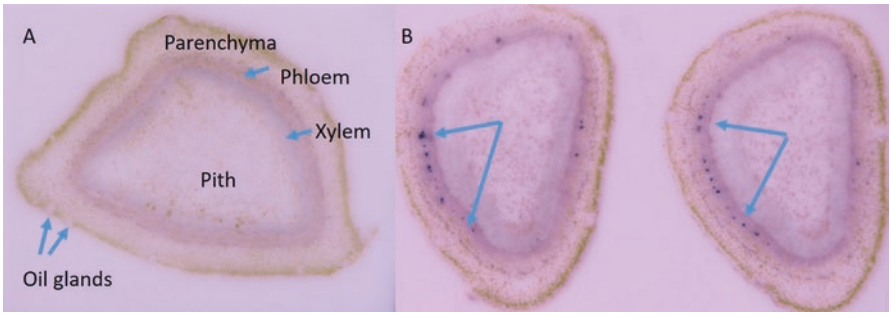

Fig. 10.1 Immune tissue print of cross sections of citrus stems from (**a**) healthy and (**b**) CLas-infected trees. Labels and arrows in A identify anatomical features. Arrows in B point to phloem cells that are identified as filled with CLas

Using the software program tensorflow and supervised machine learning, curated collections of images known to be CLas-positive or CLas-negative such as these were submitted as training sets to create trained CNNs using the Inception V3 architecture. Four trained CNNs based on images from stems and petioles were created independently. They were tested by submitting curated training sets of images to them, as unknowns. Such a set of unknowns in machine learning is called a test set. The CNNs fitted each image into the 'CLas-infected' or 'CLas-uninfected' models and produced a probability of fit to each model, to estimate the probability that each tissue-print image presented an image from a CLas-infected or an uninfected tree. If perfect, the model would agree with the curator with 100% confidence. The model trained on images of petioles gave better results overall than did the model based on images of stems, with 71% vs 64% of images correctly categorized with more than 90% confidence, and 95% vs 80% of images correctly categorized with more than 80% confidence (Table 10.1 Model 3).

A new CNN was created as before for images of petioles, with the training set adjusted by removing images of petioles that the curator considered to be somewhat atypical of what could be expected in CLas-positive or CLas-negative petioles. This adjustment to the training set only slightly improved the agreement of the categorization of the tissue prints between the curator and the petiole model, with 81% of the CLas-positive and CLas-negative petiole images correctly categorized at >90% confidence (Table 10.1 Model 4). The results of the stem and petiole model were also summarized graphically (Fig. 10.2). Using this model, a minimum of 64% of

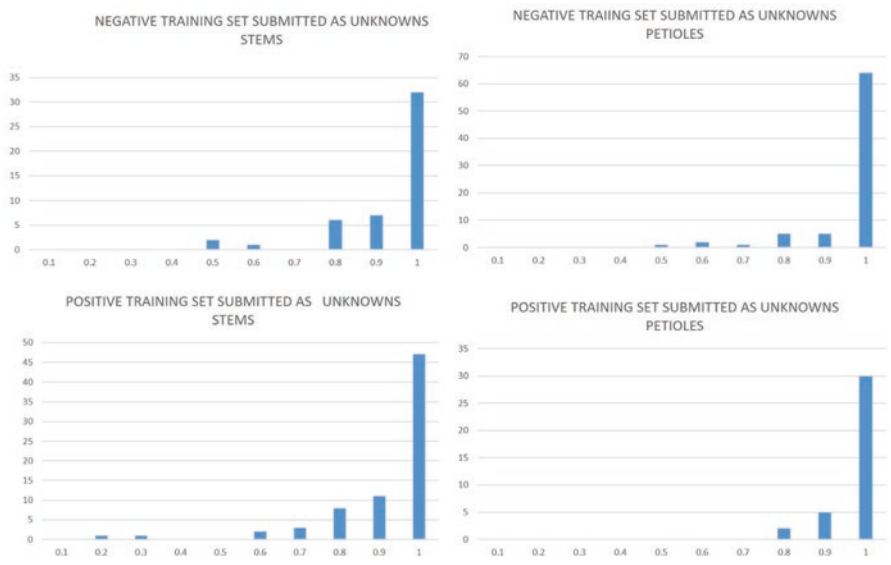

Fig. 10.2 Summary of results of models for the classification of images of stems and petioles of citrus plants as CLas-infected or uninfected. The curated images used to train the model were submitted to the model as unknowns. The upper limit of the confidence interval is given on the X-axis and the number of images in each confidence interval is given on the Y-axis

all images were classified in agreement with the curator with more than 90% confidence, and 90–100% of all images were classified in agreement with the curator with more than 70% confidence (Table 10.2).

The evaluation by the models of individual images not used previously to train the models reveal how they are working. Three images of uninfected (Fig. 10.3a–c) and three images of CLas-infected petioles (Fig. 10.3d–f) show that the model agreed with the curator with greater than 95% confidence. Image 3B was evaluated as uninfected by both the curator and the model, but with only 80% confidence. This is likely due to several dark spots appearing in the xylem tissue of the image, where CLas is not expected to occur. Evidently the model took these ectopic spots into account and produced a lower confidence score in an otherwise correct decision.

Similar results were produced by the stem model (Fig. 10.4). Five of six stem images of CLas-infected (Fig. 10.4g–i) and uninfected stems (Fig. 10.4j–l) were evaluated by the stem model in agreement with the curator with greater than 93% confidence. Image 3L was evaluated in agreement with the curator, but with only 81% confidence. A very typical CLas spot was present in the phloem of Fig. 10.3l, as in Fig. 10.3j, but there were also large numbers of spots outside the phloem, in the periderm, where CLas is not expected to be found. The model evidently evaluated these ectopic spots as atypical, and returned a call with only 81% confidence that image 3L was of a CLas-infected sample.

Overall, both the petiole and stem models agreed with the curator on the infection status of tissue prints prepared from either petiole or stem sections of plants. Chi-square tests without Yates correction were significantly different from random assignment of classification ($P < 0.001$) when the confidence level of the category assignment was greater than 50% (Table 10.3).

There were two instances where the images were incorrectly classified by the model (Fig. 10.5). These images were selected by the curator as very typical CLas-infected tissue prints prepared from stem sections and were included in the training set, but when the images were presented to the models they were classified as CLas-infected with only 16% and 26% confidence (Fig. 10.5), meaning they were incorrectly classified as uninfected with 84% and 74% confidence.

Table 10.2 Evaluation of CLas petiole and stem models for the detection of CLas in phloem cells using images of trees either infected with CLas or free of CLas that were not used to train the model

Petiole 2 × 2 contingency table[a]				Stem 2 × 2 contingency table[b]			
	pp	pn	Total		pp	pn	Total
P[c]	62	3	65	P	129	46	175
N	0	32	32	N	10	23	33
Total	62	35	97	Total	139	69	208

[a]Chi-square without Yates correction equaled 84.6 with 1 df. Two-tailed $P < 0.0001$

[b]Chi-square without Yates correction equaled 23.6 with 1 df. Two-tailed $P < 0.0001$

[c]P, N Images classified as positive or negative for Clas by the curator; pp., pn Images classified as positive or negative for Clas by the CNN model

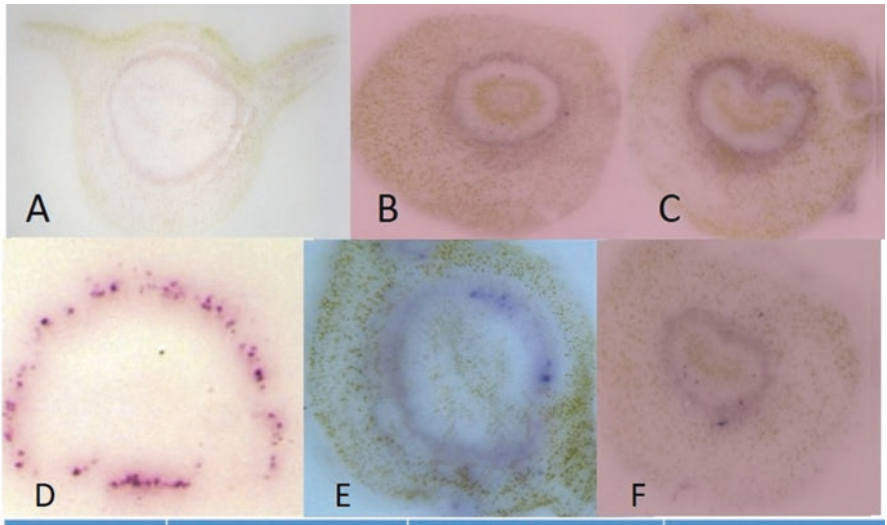

Image	Curator	Clas-Infected	Uninfected
A	Uninfected	0.045	0.955
B	Uninfected	0.197	0.803
C	Uninfected	0.043	0.957
D	Clas-Infected	0.955	0.045
E	Clas-Infected	0.992	0.008
F	Clas-Infected	0.977	0.023

Fig. 10.3 Immune tissue-print of cross sections of uninfected (**A**-**C**) and CLas-infected (**D**-**F**) stems not used to train the model. The inset gives the probabilities calculated by the model that the images are from CLas-infected or uninfected petioles

Discussion

When CLas/HLB is thought to be absent in an area, it is wise to sample the psyllid population for CLas, because it has been shown that detection of the pathogen in psyllids precedes detection of the pathogen in trees (Keremane et al. 2008). However, once the pathogen has been detected in an area, especially vigorous sampling and

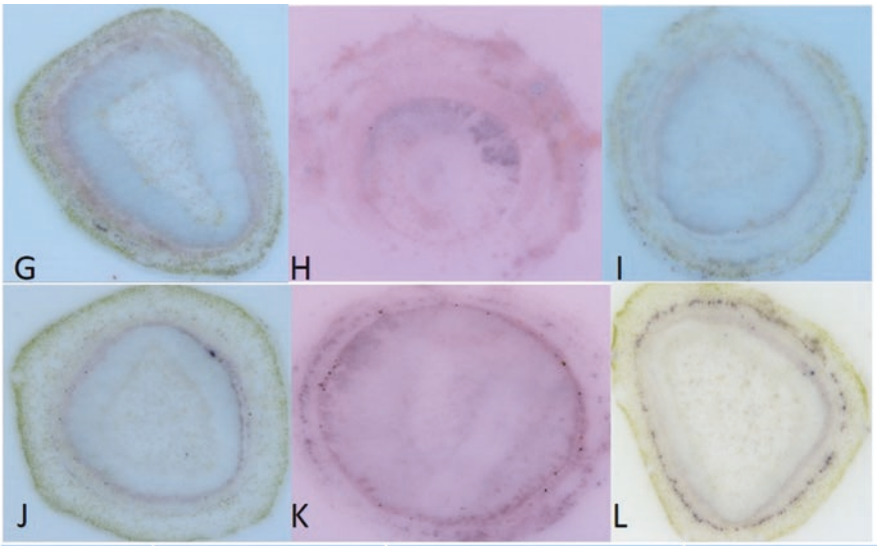

Image	Curator	Clas-Infected	Uninfected
G	Uninfected	0.063	0.937
H	Uninfected	0.040	0.960
I	Uninfected	0.007	0.993
J	Clas-Infected	0.937	0.063
K	Clas-Infected	0.951	0.048
L	Clas-Infected	0.812	0.188

Fig. 10.4 Immune tissue-print of cross sections of uninfected (G-I) and CLas-infected (J-L) stems not used to train the model. The inset gives the probabilities calculated by the model that the images are from CLas-infected or uninfected stems

removal of infected trees is required to limit the ability of the psyllid to acquire CLas inoculum for further local exponential transmission. This is typically done by laborious and expensive qPCR-based methods to test trees with symptomatic tissues (Anonymous 2014; Li et al. 2006). As shown by the history of the epiphytotic in Florida and elsewhere, testing for CLas after symptoms have appeared is ineffective, because asymptomatic but infected trees convert to symptomatic trees in near

Table 10.3 Confidence intervals associated with classification of images as CLas-infected or uninfected by stem and petiole models when curated images used in the training set were submitted to the model as unknowns

Classification by Curator	Confidence interval of model identification	Number/per cent of images in confidence interval
STEM NEGATIVES	>90	32/67%
	>80	7/81%
	>70	6/94%
Total images submitted		48
STEM POSITIVES	>90	49/64%
	>80	11/80%
	>70	8/90%
Total images submitted		73
PETIOLE NEGATIVES	>90	64/82%
	>80	5/89%
	>70	5/95%
Total images submitted		78
PETIOLE POSITIVES	>90	30/81%
	>80	5/95%
	>70	2/100%
Total images submitted		37

unison (Gottwald et al. 2007). For this reason several groups have attempted to develop alternative means to test asymptomatic citrus trees for the presence of CLas (Arredondo Valdés et al. 2016; Chin et al. 2014; Pagliaccia et al. 2017).

We have developed the tissue-print format, which has several advantages over PCR-based methods for detection of CLas, because DNA extraction and amplification reagents and materials are not needed, and the tissue prints themselves do not require laboratory facilities to be prepared. The assay scales to large numbers of samples at very little cost, once the antibody is available. The serologically based tissue-print assay is also an ideal complement to qPCR testing and can be performed concurrently with a qPCR assay to provide mutually confirmatory results (Fu et al. 2019).

Two CNN models were developed to classify images from either petioles or stem sections of citrus trees. The models return a value for probability of a match to both models (CLas-infected or uninfected) with calculated confidence values that sum to 1. These confidence values are a major advantage of the automated approach and cannot be provided by the human expert, who can only provide a qualitative and binary classification. The models worked very well, with the hypothesis of a random assignment to one class or the other rejected ($P < 0.0001$) and with 90% of curated samples identified in agreement with the expert curator.

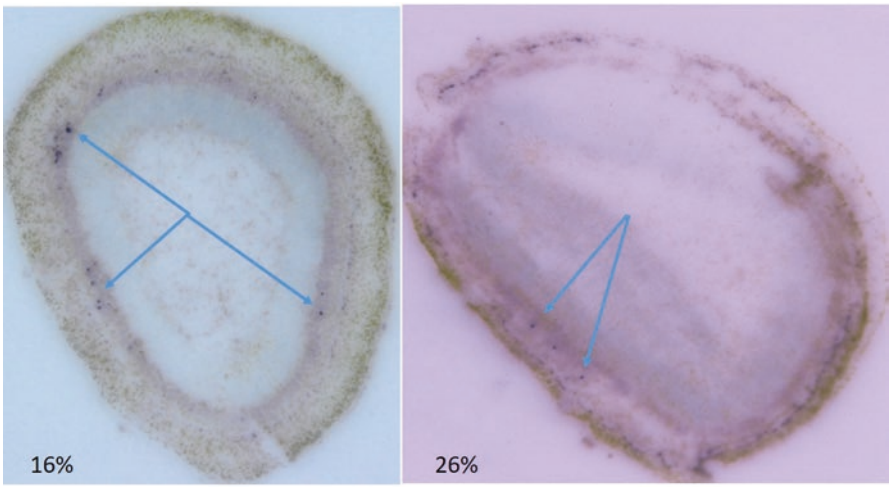

Fig. 10.5 Two images of stem sections that the curator classified as CLas-infected but the model classified as CLas infected with only 16% and 26% confidence (incorrect classification). Arrows indicate colored spots in the phloem tissue that are entirely typical of CLas-infected tissue prints

A large number of samples is required for testing because of the large number of leaves on a citrus tree, the large number of trees in a grove, and the notoriously erratic distribution of the pathogen even when symptoms are present (Li et al. 2009; Tatineni et al. 2008). Because of the erratic distribution of the pathogen and large number of leaves and trees that must be evaluated, neither qPCR nor immune tissue-printing is adequate for large-scale testing of citrus groves. For this purpose, several technologies are being developed, including chemical analysis of the volatile and metabolic components produced by the trees (i.e. the volitome, metabolome) (Chin et al. 2014; Slisz et al. 2012), hyperspectral imaging of the canopy of trees to detect pathogen-induced changes not visible to the human eye (Kumar et al. 2012), and the use of canines trained to detect CLas as they are commonly trained to detect explosives and agricultural commodities at airports (Gottwald et al. 2017). The latter technique is especially promising since the dogs can rapidly survey a grove and reliably detect trees that are infected and asymptomatic and would otherwise not be suspected of being infected (Gottwald et al. 2020). Confirmatory tests of trees identified by these methods are still desirable. Trees identified by any of these novel, whole-tree-based methods could be exhaustively sampled by immune tissue printing, which has been extensively validated (Ding et al. 2015, 2016) with automated classification at much lower cost than by qPCR. In addition, the immune tissue printing assay can be performed concurrently with qPCR, with the important advantage of eliminating the expensive and laborious process of DNA extraction and purification typically needed prior to qPCR, by the simple expedient of eluting the DNA directly from the nitrocellulose membrane used to prepare the tissue print (Bertolini et al. 2014; Fu et al. 2019).

Although the preparation of immune tissue prints is relatively simple to do and allows high throughput, the image acquisition requires a digital microscope. Suitable microscopes are available at low cost. The classification models do however require a substantial investment in computational resources. We plan to make the models available on the web so that users could submit limited numbers of images for classification. Alternatively, we could provide the models upon request to users with the computational infrastructure and expertise to run the classifiers in batch mode. We did not perform any post-acquisition processing of the images to make them uniform in terms of centering or orientation or color correction. This takes advantage of the translationally invariant property of CNNs and makes the models robust and user-friendly.

In image classification with convolutional neural networks, each section of the image being classified is made up of thousands of pixels. Pixels at the edges of salient features are especially important. It is interesting to note that computer hackers can train a CNN to recognize images of objects, and then make subtle adjustments to individual pixels at the edges of significant features in an individual image so that the CNN makes an incorrect classification of the image. The changes in the pixel values introduced by the hacker are imperceptible to a human viewer but are sufficient to entirely fool the model (Geitgey 2017). Edge effects in the images submitted to our models for classification but which were imperceptible to the curator may explain the occasional failure of the models to agree with the curator (Fig. 10.5). Image analysis using convolutional or deep neural networks still has challenges to overcome and still requires a degree of human review.

Others have developed automated image analysis environments through machine learning to classify plants according to phenotype (Hartmann et al. 2011; Minervini et al. 2015) and to identify symptoms associated with particular plant diseases (Mutka and Bart 2015). Analysis of the plant phenotype or symptoms is however not useful for the management of HLB, because the disease has an extended cryptic stage in which symptoms are not visible but during which the pathogen can be acquired and mitted by the insect vector. Thus it was necessary to develop novel models such as those described here.

There are other diseases of citrus in which the pathogen has a phloem-limited distribution like CLas, for example *Spiroplasma citri* (citrus stubborn) (Yokomi et al. 2008) and many plant viruses notably the Closterovirus citrus tristeza virus (tristeza) (Dawson et al. 2015) and the exotic Begomovirus citrus chlorotic dwarf virus (Loconsole et al. 2012). As suitable primary antibodies become available, the computational models developed for classification of immune tissue prints for the detection of CLas should be immediately useful for these pathogens as well.

Acknowledgments This research used resources provided by the SCINet project of the USDA Agricultural Research Service, ARS project number 0500-00093-001-00-D.

References

Anonymous. (2014). PM 7/121 (1) 'Candidatus Liberibacter africanus', 'Candidatus Liberibacter americanus' and 'Candidatus Liberibacter asiaticus'. *Bulletin OEPP/EPPO Bulletin, 44*(3), 376–389.

Arredondo Valdés, R., Delgado Ortiz, J. C., Beltrán Beache, M., Anguiano Cabello, J., Cerna Chávez, E., Rodríguez Pagaza, Y., & Ochoa Fuentes, Y. M. (2016). A review of techniques for detecting Huanglongbing (greening) in citrus. *Canadian Journal of Microbiology, 62*(10), 803–811.

Bertolini, E., Felipe, R. T. A., Sauer, A. V., Lopes, S. A., Arilla, A., Vidal, E., Mourão Filho, F. A. A., Nunes, W. M. C., Bové, J. M., López, M. M., & Cambra, M. (2014). Tissue-print and squash real-time PCR for direct detection of '*Candidatus* Liberibacter' species in citrus plants and psyllid vectors. *Plant Pathology, 63*, 1149–1158.

Bové, J. M. (2006). Huanglongbing: A destructive, newly-emerging, century old disease of citrus. *Journal of Plant Pathology, 88*, 7–37.

Cassab, G. I., & Varner, J. E. (1987). Immunocytolocalization of extensin in developing soybean seed coats by immunogold-silver staining and by tissue printing on nitrocellulose paper. *The Journal of Cell Biology, 105*(6), 2581–2588.

Chin, E. L., Mishchuk, D. O., Breksa, A. P., & Slupsky, C. M. (2014). Metabolite signature of *Candidatus* Liberibacter asiaticus infection in two citrus varieties. *Journal of Agricultural and Food Chemistry, 62*(28), 6585–6591.

Dawson, W. O., Bar-Joseph, M., Garnsey, S. M., & Moreno, P. (2015). *Citrust tristeza virus*: Making an ally from an enemy. *Annual Review of Phytopathology, 53*, 137–155.

Deng, H., Achor, D., Exteberria, E., Yu, Q., Du, D., Stanton, D., Liang, G., & Gmitter, F. G., Jr. (2019). Phloem regeneration is a mechanism for Huanglongbing- tolerance of "Bearss" lemon and "LB8-9" Sugar Belle® Mandarin. *Frontiers in Plant Science, 10*(277).

Ding, F., Duan, Y., Paul, C., Brlansky, R. H., & Hartung, J. S. (2015). Localization and distribution of 'Candidatus Liberibacter asiaticus' in citrus and periwinkle by direct tissue blot immuno assay with an anti-OmpA polyclonal antibody. *PLoS One, 10*(5), e0123939.

Ding, F., Duan, Y., Yuan, Q., Shao, J., & Hartung, J. S. (2016). Serological detection of '*Candidatus* Liberibacter asiaticus' in citrus, and identification by GeLC-MS/MS of a chaperone protein responding to cellular pathogens. *Scientific Reports, 6*, 29272.

Duan, Y., Zhou, L., Hall, D. G., Li, W., Doddapaneni, H., Lin, H., Liu, L., Vahling, C. M., Gabriel, D. W., Williams, K. P., Dickerman, A., Sun, Y., & Gottwald, T. (2009). Complete genome sequence of citrus huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus' obtained through metagenomics. *Molecular Plant-Microbe Interactions, 22*(8), 1011–1020.

Fu, S. M., Liu, H. W., Liu, Q. H., Zhou, C. Y., & Hartung, J. S. (2019). Detection of '*Candidatus* Liberibacter asiaticus' in citrus by concurrent tissue print-based qPCR and immunoassay. *Plant Pathology*, 68, 796–803.

Geitgey, A. (2017). *Machine learning is fun part 8: How to intentionally trick neural networks*. Machine Learning is Fun. https://medium.com/@ageitgey/machine-learning-is-fun-part-8-how-to-intentionally-trick-neural-networks-b55da32b7196. Retrieved 7/3/2019.

Gottwald, T. R., & McCollum, T. G. (2017). Huanglongbing solutions and the need for anti-conventional thought. *Journal of Citrus Pathology, 36952*(4), 1.

Gottwald, T. R., da Graça, J. V., & Bassanezi, R. B. (2007). Citrus Huanglongbing: The pathogen and its impact. *Plant Health Progress*. https://doi.org/10.1094/PHP-2007-0906-01-RV.

Gottwald, T. R., Poole, G. H., Taylor, E. L., Hartung, J. S., Hall, D. G., Bartels, D., McCollum, T. G., Hilf, M. E., Luo, W., Louws, F., Polek, M., Vidalakis, G., & Mauk, P. (2017). Use of HLB detection canines in real world settings, 5th International Research Conference on Huanglongbing, Orlando, FL. *Journal of Citrus Pathology, 4*, 16. https://escholarship.org/uc/item/2cr0f2kc.

Gottwalda, T., Poolea, G., McColluma, T., Halla, D., Hartung, J., Baia, J., Luoa, W., Posnya, D., Duana, Y-P., Taylora, E., da Graçad, J., Poleke, M., & Schneiderf, W. (2020). Canine olfactory

detection of a vectored phytobacterial pathogen, Liberibacter asiaticus, and integration with disease control. *PNAS, 117*(7): 3492–3501.

Graham, J. H., Johnson, E. G., Gottwald, T. R., & Irey, M. S. (2013). Presymptomatic fibrous root decline in citrus trees caused by huanglongbing and potential interaction with *Phytophthora* spp. *Plant Disease, 97*, 1195–1199.

Grosser, J. W., Gmitter, F. G., & Castle, W. S. (2016). Breeding citrus rootstocks to mitigate Huanglongbing (HLB, or citrus greening disease). *Acta Hort, 1127*, 83–88.

Hartmann, A., Czauderna, T., Hoffmann, R., Stein, N., & Schreiber, F. (2011). HTPheno: An image analysis pipeline for high-throughput plant phenotyping. *BMC Bioinformatics, 12*, 148.

Hartung, J. S., Paul, C., Achor, D., & Brlansky, R. H. (2010). Colonization of dodder, *Cuscuta indecora,* by '*Candidatus* Liberibacter asiaticus' and '*Ca*. L. americanus. *Phytopathology, 100*(8), 756–762.

Keremane, M. L., Halbert, S. E., Ramadugu, C., Webb, S., & Lee, R. F. (2008). Detection of *Candidatus* Liberibacter asiaticus in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology, 98*(4), 387–396.

Knighten, C., Redding, J., Feiber, D., & Compton, L. (2005). *U.S. Department of Agriculture and Florida Department of Agriculture confirm detection of citrus greening. Department Press Release: 30*.

Kumar, A., Lee, W. S., Ehsani, R. J., Albrigo, L. G., Yang, C., & Mangan, R. L. (2012). Citrus greening disease detection using aerial hyperspectral and multispectral imaging techniques. *Journal of Applied Remote Sensing, 6*(1), 063542.

Li, W., Hartung, J. S., & Levy, L. E. (2006). Quantitative real time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. *J. Microbiological Methods, 66*, 104–115.

Li, W., Levy, L., & Hartung, J. S. (2009). Quantitative distribution of '*Candidatus* Liberibacter asiaticus' in citrus plants with citrus huanglongbing. *Phytopathology, 99*(2), 139–144.

Liu, H., Atta, S., & Hartung, J. S. (2017). Characterization and purification of proteins suitable for the production of antibodies against '*Ca*. Liberibacter asiaticus. *Protein Expression and Purification, 139*, 36–42.

Loconsole, G., Saldarelli, P., Doddapaneni, H., Savino, V., Martelli, G. P., & Saponari, M. (2012). Identification of a single-stranded DNA virus associated with citrus chlorotic dwarf disease, a new member in the family Geminiviridae. *Virology, 432*, 162–172.

Lyle, S. (2012). *Citrus disease huanglongbing detected in Hacienda Heights area of Los Angeles County* (pp. 12–012). Sacramento: California Department of Food and Agriculture.

Lyle, S. (2015). *Citrus disease huanglongbing detected in the San Gabriel aera of Los Angeles county* (Vol. 1, pp. 15–031). Sacramento: California Department of Food and Agriculture.

Minervini, M., Scharr, H., & Tsaftaris, S. A. (2015). Image analysis: The new bottleneck in plant phenotyping. *IEEE Signal Processing Magazine, 32*(7123050), 126–131.

Morgan, J. K., Zhou, L., Li, W., Shatters, R. G., Keremane, M., & Duan, Y. P. (2012). Improved real-time PCR detection of Candidatus Liberibacter asiaticus from citrus and psyllid hosts by targeting the intragenic tandem-repeats of its prophage genes. *Molecular and Cellular Probes, 26*, 90–98.

Mutka, A. M., & Bart, R. S. (2015). Image-based phenotyping of plant disease symptoms. *Frontiers in Plant Science, 5*, 734.

Nath, D., & Husain, M. A. (1927). The citrus psylla (*Diaphorina citri,* Kuw.) [Psyllidae:Homoptera]. *Memoirs of the Department of Agriculture in India, 10*(2), 1–27.

Pagliaccia, D., Shi, J., Pang, Z., Hawara, E., Clark, K., Thapa, S. P., De Francesco, A., Liu, J., Tran, T.-T., Bodaghi, S., Folimonova, S. Y., Ancona, V., Mulchandani, A., Coaker, G., Wang, N., Vidalakis, G., & Ma, W. (2017). A pathogen secreted protein as a detection marker for citrus huanglongbing. *Frontiers Microbiology, 8*, 2014.

Slisz, A. M., Breksa, I., Andrew, P., Mishchuk, D. O., McCollum, G., & Slupsky, C. M. (2012). Metabolomic analysis of citrus infection by '*Candidatus* Liberibacter' reveals insight into pathogenicity. *Journal of Proteome Research, 11*, 4223–4239.

Stewart, M. (2019). Simple introduction to convolutional neural networks. *Towards Data Science.* https://towardsdatascience.com/simple-introduction-to-convolutional-neural-networks-cdf8d3077bac?gi=c67d57bc819f. Retrieved 7/3/2019.

Tatineni, S., Shankar Sagaram, U., Gowda, S., Robertson, C. J., Dawson, W. O., Iwanami, T., & Wang, N. (2008). In planta distribution of '*Candidatus* Liberibacter asiaticus' as revealed by polymerase chain reaction (PCR) and real-time PCR. *Phytopathology, 98*(5), 592–599.

Wang, N., & Trivedi, P. (2013). Citrus huanglongbing: A newly relevant disease presents unprecedented challenges. *Phytopathology, 103*, 652–665.

Yokomi, R. K., Mello, A. F. S., Saponari, M., & Fletcher, J. (2008). Polymerase chain reaction-based detection of *Spiroplasma citri* associated with citrus stubborn disease. *Plant Disease, 92*, 253–260.

Zheng, Z., Xu, M., Bao, M., Wu, F., Chen, J., & Deng, X. (2016). Unusual five copies and dual forms of nrdB in "*Candidatus* Liberibacter asiaticus": Biological implications and PCR detection application. *Scientific Reports, 6*, 39020.

Chapter 11
Plantwise: A Knowledge and Intelligence Tool for Food Security through Crop Protection

Claire Beverley and Manju Thakur

Abstract Food security continues to be significantly impacted by a growing world population, changing climate, increasing food prices and environmental burden. One of the key challenges in reducing crop losses due to pests and diseases is timely delivery of appropriate, actionable extension advice to farmers. Information and communication technology (ICT) has the potential to improve services that connect smallholder farmers to new resources and information, helping to build their knowledge and ultimately improve their livelihoods. Such ICT-driven services have seen rapid growth over the past few years, and CABI has been harnessing this technology in several programmes. This chapter provides insight into digital interventions of the global, CABI-led programme, Plantwise, which aims to assist stakeholders in developing countries to improve their plant health systems by strengthening linkages among all actors involved, so that they can prevent and manage pest outbreaks more effectively. An overview of digital interventions piloted and tested under the umbrella of the Plantwise programme is illustrated with selected case studies. Interventions include pest diagnosis and management advice delivered via a website, plant health data collection, using a customized mobile application, and educational simulation games for ongoing support.

C. Beverley (✉)
CABI, Wallingford, UK
e-mail: c4beverley@hotmail.co.uk

M. Thakur
CABI, New Delhi, India
e-mail: m.thakur@cabi.org

© The Author(s) 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_11

Background

Smallholder farmers in developing countries, who produce enough food to feed their families with some surplus to sell at the local market, comprise one of the main groups to be affected by emerging plant pests[1] and subsequent yield loss. Approximately 500 million smallholder farmers provide over 80% of the food for a large part of the developing world (International Fund for Agricultural Development 2013). CABI, through its flagship development programme, Plantwise, aims to support relevant United Nations Sustainable Development Goals (SDGs) by improving farmers' yields and incomes, while reducing the use of toxic pesticides through the provision of actionable management advice based on safe principles of integrated pest management (IPM).

Achieving a world without hunger by 2030 depends on increasing the productivity of smallholder farmers; however, their crops face significant threats. Yearly, an estimated 25–40% of six major crops grown worldwide is lost to pests (Oerke 2006), and yield loss estimates recorded by Savary et al. (2019) suggest the highest losses are associated with food-deficit regions with fast-growing populations, and frequently with emerging or re-emerging pests and diseases. If crop losses were reduced by as little as 1%, millions more people could be fed (Plantwise 2019) . The challenge is to deliver appropriate, actionable extension advice to farmers, at the right time, to help them reduce crop losses.

Plantwise is a global programme led by CABI to help stakeholders in developing countries improve their plant health systems by strengthening linkages between stakeholders, so that they can prevent and manage pest outbreaks more effectively. The focus of Plantwise is to support male and female smallholder farmers in developing countries to lose less of what they grow to pests so that food security is increased, poverty is alleviated and livelihoods are improved. A global network of plant clinics in more than 30 developing and transitional countries across Africa, Asia and the Americas has been established in collaboration with national agricultural advisory services. Plantwise enables farmers to manage pests more effectively by improving the creation, quality and exchange of plant health knowledge.

The plant clinic concept, which is based on the human healthcare system (Bentley 2009), first began in an initiative called the Global Plant Clinic (Boa 2009), and is now promoted through Plantwise. The plant clinics are owned and run by plant health extension staff who have been trained as plant doctors. Plant doctor training, developed by science experts at CABI, comprises two modules covering field diagnostics and plant clinic operation (module 1) and giving good recommendations (module 2). Training is delivered either by CABI staff or by partners within the agricultural advisory services that have taken part in plant doctor training and a subsequent Training of Trainers (TOT) programme. Once training is complete, plant doctors are able to set up demand-driven plant clinics where farmers can seek

[1] Definition from IPPC in ISPM5 "Any species, strain or biotype of plant, animal or *pathogenic* agent injurious to plants or plant products" (FAO 2013).

free, practical and immediate crop management advice. Farmers visit plant health clinics with samples of their affected crops, and plant doctors offer a science-based diagnosis followed by a recommendation of how to manage the current problem and advice on ways to prevent it in the future.

The plant clinic network is reinforced by the Plantwise Knowledge Bank, a gateway to practical, online and offline plant health information, including diagnostic resources and best-practice pest management advice.

Harnessing Innovative Technologies for Information Delivery and Collection

Online Plantwise Knowledge Bank

Through the Plantwise programme, CABI has harnessed different information and communication technology (ICT) approaches to reach out to key stakeholders. The first and foremost intervention in this direction was the launch of the Plantwise Knowledge Bank website in 2012. It was recognized early on in the programme that there would be a need for a central information resource for crop protection, the Knowledge Bank, to serve the diverse stakeholders within a national plant health system (Leach and Hobbs 2013).

The Plantwise Knowledge Bank (www.plantwise.org/knowledgebank) is an open-access, online resource that provides plant health information to users, with focus on developing and transitional countries across Africa, Asia and the Americas. Core publishing capabilities of CABI are supplemented with the knowledge, skills and understanding of internationally based science staff and their network of collaborators to deliver science-based information for use in local contexts. The gateway aims to deliver dependable information serving users with widely differing needs and understanding, and to deliver actionable knowledge to subsistence farmers in particular.

This freely available resource brings together over 10,000 factsheets covering more than 6500 host plants and pests in over 80 languages. Factsheets include Pest Management Decision Guides and Plantwise Factsheets for Farmers, produced as part of the programme. Creating a format for pest management advice that is of practical use to smallholder farmers is a major challenge (Katherine et al. 2016; Chundi 2014; Masuki et al. 2011; Sones et al. 2015); therefore, CABI and its partners use models for pest management content that are easy to understand, containing effective, safe and practical advice for the local context. Plantwise Factsheets for Farmers (Bentley and Boa 2013) and Pest Management Decision Guides (Chernoh and Kuhlmann 2015) are developed in partnership with local experts. The materials are written in local languages and provide a range of IPM options, illustrated with images of pests to aid diagnosis. Plantwise Factsheets are reviewed by local farmers in the countries where they are written to ensure that the information

is easy to understand and practical to use. They are validated by technical reviewers to check that the recommendations are safe and follow sound scientific principles. The Plantwise Knowledge Bank website, which has received over 2 million cumulative visits to date, provides diagnostic resources, best practice pest management advice and plant clinic data analysis for targeted crop protection (Leach and Hobbs 2013; Katherine et al. 2016).

The Country Resources section of the website contains country-specific resources including pesticide lists, diagnostic support and guidelines for writing extension materials. It is in this section that visitors will find the Plantwise Diagnostic Field Guide, which supplements training modules 1 and 2 on 'How to become a Plant Doctor' (Taylor 2015) (https://www.plantwise.org/diagnostic-field-guide/). The guide provides images and descriptions of many typical symptoms as well as 'ready reckoners', which are simple information tables for quick and easy reference, describing relationships between common symptoms on plants and the various possible causes. The guide also provides a short overview of the important principles for giving good advice, which are underpinned by an IPM approach.

Plantwise Online Management System

While the Plantwise Knowledge Bank is referred to as 'open access', the Plantwise Online Management System (POMS) is often referred to as the 'closed access' section of the Knowledge Bank. The POMS is a secure, bespoke, central data management system owned and maintained by CABI for managing plant clinic and administrative data necessary for the maintenance and analysis of clean information. Data contained within the POMS is only accessible via a username and password, and is owned by the country of origin.

When the plant clinics were first piloted under the Global Plant Clinic initiative, advisers began recording some basic information about the farmers who visited the clinics, their crops and problems, and the advice being given (Bentley 2009). The potential value of the data was recognised and CABI subsequently worked with partners to standardise data collection in each of the countries where Plantwise was operating.

Plant Clinic Data Collection

The interaction between a plant doctor and a farmer at a plant clinic is recorded on a prescription form, which is available as paper or electronically in a Data Collection application (DCA), for use on a phone or tablet. The record of each interaction is referred to as a clinic query. Those clinics operating with the electronic version of the prescription form are referred to as e-plant clinics. Information collected on

paper forms is digitised using a desktop version of the DCA and all clinic queries, irrespective of form type, are collated in the POMS.

The prescription form takes a plant doctor through an 'interview' with a farmer, using the principles of IPM, to diagnose plant health problems and provide a suitable and safe recommendation either on a paper copy of the form or via a short message service (SMS) message sent directly to the farmer's phone. In e-plant clinics, a plant doctor is unlikely to have internet connection during clinic sessions; therefore, the DCA works offline for forms to be filled in and saved. When the plant doctor has sufficient internet coverage and/or mobile data, the forms can be submitted to the POMS. The app allows plant doctors to send SMS messages containing high quality advice to farmers, while quickly and easily collecting plant health data on crop grown, problem description, diagnosis and recommendation, during their regular clinics and farm visits. The app also includes a report feature so that plant doctors can keep track of how many farmers they have helped. This function enables them to see trends in country data and to quickly and easily update their supervisors on the number of farmers they have reached.

Plantwise tested the use of ICT (tablets and SMS) with 60 Kenyan extension workers during a 1-year pilot. Results indicated that extension workers were able to assist more farmers with better advice, had significantly improved access to plant health information, valued being able to ask their peers for advice, and dramatically improved the quality and speed of the data they collected (Wright et al. 2016). To date, over 3,953 plant doctors have been trained on the use of tablet computers at plant clinics in 28 countries.

The speed with which data are collected and the opportunities this creates for informing plant health services of current issues on the ground make e-plant clinics the preferred method of clinic service delivery.

Plant clinics operate in varied country contexts, but the Plantwise programme encourages the following operational guidelines to ensure that farmers' interests remain at the heart of service delivery:

- Plant clinics should accept any crop and any type of problem;
- Plant clinics should be open to all farmers and aim to provide equal access to men and women from all social and ethnic groups;
- Plant clinics should operate in a visible and accessible location, ideally somewhere that farmers go regularly, to minimise the effort and cost of facilitating an opportunity for face-to-face dialogue;
- Plant clinics should operate on a regular, or at least predictable, basis and at times that are convenient to farmers. Many of the clinics that have been established through Plantwise only run periodically, such as for a few hours per week or even less frequently. The periodicity depends on the availability and priorities of the extension staff; thus, a certain amount of publicity is typically required to let farmers know of the next clinic session (Jenner et al. 2020).

Because clinics are inclusive and offer advice on any crop and type of problem, plant doctors need to have basic plant health knowledge. They can supplement this with information in the Plantwise Factsheets Library app (see Offline Plantwise

Knowledge Bank); the DCA was built to work well in combination with this app. Users are encouraged to switch between the apps, when needed, and even copy suitable recommendations from factsheets in the library and paste into the recommendation field of the DCA, which is used to create the SMS message. The DCA offers the functionality to collect data on the recommendation in one language, for country-wide analyses once the prescription form has been submitted to the central database, while sending an SMS message to the farmer in the local language. Approximately 1100 SMS messages were sent over the past year.

The DCA, although free to download, will only work for plant doctors with registered accounts and requires a username and password to log in. There are now 728,850 plant clinic queries in the POMS in the central database, submitted from 28 countries since inception in 2015, with nearly 60% submitted via the DCA and desktop version of the DCA.

Clinic queries that are submitted to the POMS are owned by national partners; therefore, during the process of setting up Plantwise in a country, a representative of the National Implementing Organization is required to sign a Data Sharing Agreement, countersigned by a representative of CABI, which outlines the terms of agreement for holding and using the data. Agreements can either be open (allowing CABI to hold the data in the POMS and publish on the globally accessible platform of the Plantwise Knowledge Bank) or closed (allowing CABI to hold the data, but not publish it). The whole process of managing clinic queries (data) is referred to as data management, and country partners undergo training, created by CABI, to understand the process and the importance of collecting and using data.

The National Data Manager (often in collaboration with the National Coordinator), who is responsible for activities relating to data management, determines who has access to the data from the country concerned. The National Data Manager adheres to Terms of Reference that state "Identified by the National Responsible Organization, and working closely with other Plantwise stakeholders, the National Data Manager (NDM) will have the remit for the collection, processing, storage, distribution and management of plant clinic data for a country. They will also, in collaboration with local partners, oversee the implementation of the data management processes according to the country-specific yearly plans of operation."

In order to unlock the potential value of the clinic data, a key goal of the Plantwise programme is to make the clinic data as easily accessible and reusable as possible, and therefore tools within the POMS to clean and analyse data have been developed. A harmonization tool allows users (usually NDMs) to clean data submitted by the plant doctors and develop country-specific libraries of terms for ease of management. Once clinic queries are harmonized and submitted to the live system of the POMS they are available for users to interrogate online and offline. Data interrogation is enabled using data visualisation tools in the POMS or by choosing one of five download options facilitating offline analysis in Excel or statistical packages such as R. Plantwise is committed to providing online and offline tools for accessing and using information, and has developed an offline version of the data analysis tool. Data visualisation tools allow users to identify common and unusual pest

occurrences, spread of new pests and pest outbreaks. The system provides mechanisms to analyse plant clinic data for targeted crop protection. Data collected in the POMS act as a feedback mechanism to identify problems in the field and provide targeted advice to farmers, e.g. for mass extension campaigns, identification of topics for the development of relevant extension materials, etc. In this way, plant doctors will be armed with the most relevant resources to help farmers combat pest problems.

Offline Plantwise Knowledge Bank

Plantwise Factsheets Library App

Advances in ICT and the increased popularity and accessibility of apps created an opportunity to provide another mechanism for accessing high quality plant health information. The Plantwise Factsheets Library app, launched in 2014, enables free, easy access to the most up-to-date, relevant material with the safest advice in the form of Plantwise Factsheets for Farmers and Pest Management Decision Guides. Plant doctors with mobile phones or tablets have thousands of factsheets at their fingertips when conducting plant clinic sessions, avoiding the need to carry books and leaflets, which could quickly become outdated. Users can download country-specific factsheet packs when they have an internet connection and view those country packs offline, during remote clinic sessions. The app will periodically check the servers for updates to factsheets, enabling users to stay informed about what experts consider to be today's safest and most effective management techniques. The aim is that all main problems brought to the clinics will be covered by a relevant factsheet and that, by analysing clinic data, gaps in extension materials can be identified and filled (Katherine et al. 2016). The app has proved to be a success, with more than 15,000 users in over 150 countries viewing the app in more than 700,000 sessions to date; 95% of the usage is from countries where Plantwise operates.

Plantwise Knowledge Bank USB Card

Continuing the commitment of the Plantwise programme to deliver high quality actionable plant health information to online and offline users, the Plantwise Knowledge Bank USB card was also piloted in 2014, in several languages. It was found to be a valuable tool, especially in data validation sessions. It was developed as an offline version of the Plantwise Factsheets Library app. We developed and circulated a finite number of the USB cards in 2014, but no further batches were circulated and none of the ones in circulation have been updated since. In order for them to remain useful we would need a mechanism by which users could obtain updates easily (as with the Factsheet app).

Capacity Building for Diagnostics

Extension Service Providers

The Plantwise programme is committed to leveraging ICT to continue plant doctor training remotely, supporting plant doctors who have attended Plantwise training modules 1 and 2, and who would benefit from continued education. In 2018, CABI published an e-learning course called PestSmart E-learning Diagnostics, which is derived from Plantwise material. The PestSmart Diagnostics package focuses specifically on improving the skills and methodologies required for field-based diagnosis. It helps plant health practitioners and students to develop and improve their ability to recognise symptoms, relate them to causes, and identify what is causing the problem. Containing high-resolution images, case studies and knowledge checks, the resource helps users improve their knowledge through practical, independent learning. The package covers the main pathogen groups as well as insect pests and nutrient deficiencies.

The PestSmart E-learning Diagnostics course and the PestSmart Diagnostic Simulator, together with the Diagnostic Field Guide, make up the PestSmart Diagnostics package. The aim of the package is to fast-track the field experience of plant health professionals and students by giving them CABI's wealth of field experience in a single course.

The PestSmart Diagnostic Simulator is one of several 'serious games' that have been launched and tested as a part of the Plantwise strategy to build the capacity of in-country partners to identify and diagnose pests, and to complement conventional training offered in other Plantwise training modules. The game is available on Google Play and has been developed in four languages: French, Spanish, Swahili and Chinese. Registered plant doctors and PestSmart users test their plant pest and disease investigation and diagnosis skills through seven novice-level scenarios. The game supports and reinforces the investigation and diagnosis skills of users through engaging gameplay and real-time feedback. It utilises simulated observation, inspection and deductive reasoning skills, combined with prior plant protection knowledge, to build confidence and competence in plant pest and disease diagnosis.

Another game, the Crop Management Simulator, encourages the player – in the role of a farm advisor – to help farmers face challenges of losing up to 40% of their crops to pests and diseases, based on the principles of IPM. In this game, strategies must be developed to prevent pests and diseases from destroying a farmer's crops. A range of problems from insects and mites to fungi, bacteria and viruses need to be defeated using an arsenal of control methods. Users can choose from a range of cultural, physical, biological and chemical control methods to combat pests and diseases. All control methods have their strengths and weaknesses, and can interact with each other in helpful or harmful ways. The characteristics of hundreds of pests and diseases on many globally important crops are accurate and realistic. Seasonal timing of pest attacks is modelled on real data. Changing weather patterns help to make the challenge realistic.

Serious games have been shown to improve the learning capacity of players (Connolly et al. 2012; Vlachopoulos and Makri 2017) and the Android-based games created under the Plantwise programme help to ensure continuous engagement of users in a simulation of realistic working environments in plant clinics. The games contain realistic 3D models and scenarios so that plant doctors and extension workers can hone their diagnostic skills. Further study is required of the reliability and validity of the tool; learning effects of the simulation have not yet been conclusively investigated (Thompson et al. 2016).

Rapid and accurate identification of organisms is crucial to many research and applied outcomes. Diagnosis is a critical first step in determining the significance of suspected biosecurity threats posed by emergency plant pests (EPPs) and other invasive pests and pathogens (Thompson et al. 2011). Plant doctors are generally equipped with hand-held microscopes. USB microscopes, also known as computer microscopes or computer-connected microscopes, have been trialled in plant clinics in India. The hand-held microscopes can be plugged into a USB port on a computer or television, enabling users to examine specimens via a computer monitor or television screen. The macro lens of the USB computer microscope can touch an object to magnify it or it can be used to view objects at a short distance. The images can be saved as picture files or video films, printed and sent to diagnostic experts. Plant doctors use the technology when they are unable to diagnose a problem, and share pictures and videos with other plant doctors and experts. In India, these microscopes were found to attract more farmers, increasing plant clinic attendance, and increase the diagnostic ability of plant doctors (Thakur et al. 2016).

Farmers

Plant doctors and extension workers are important providers of agricultural information and advisory services; however, they face challenges in reaching farmers at scale due to their relatively small group size, difficulties in reaching remote farms, and low motivation and accountability (Anderson and Feder 2007; Bell 2015). ICT-based extension methods can enable broader and timely outreach to farmers, often in a cost-effective and interactive way (Saravanan et al. 2015; Toepfer et al. 2019). For example, Tambo et al. (2019) reported that participation in ICT-based extension campaigns significantly increases maize farmers' knowledge about fall armyworm and stimulates adoption of agricultural technologies and practices for its management in western Uganda. They reported that of three ICT channels tested – interactive radio, mobile SMS messages and village-based video screenings – radio has greater reach, video exerts a stronger impact on the outcome measures, and greater gains are achieved when video is complemented by radio. Exposure to multiple campaign channels yields significantly higher outcomes than exposure to a single channel. The authors concluded that complementary ICT-based extension campaigns (particularly those that allow both verbal and visual communication) hold great potential to improve farmers' knowledge and trigger behavioural changes in

the identification, monitoring and sustainable management of a new invasive pest, such as fall armyworm.

Plant Clinic Data Use

Information exchange is at the heart of Plantwise, empowering plant doctors to advise farmers on growing healthy crops using the latest plant health information, while gaining insights into what problems farmers face to facilitate rapid responses. Plantwise priorities in 2019 include improvement of clinic data use by CABI and its partners. Countries in which Plantwise operates, where it is believed there is a good relationship with a range of stakeholders and where it is expected there may be greatest benefits from improving clinic data sharing, have been identified to engage in those discussions. CABI proposes to explore, with national data custodians and partners, options for mapping pest distribution, mapping and monitoring crops grown and identifying alternatives, monitoring pesticide (or other input) recommendations, monitoring service quality and 'certification', etc. Various stakeholders, including regulators, quarantine officers, agro-input manufacturers, policy makers and farmers can benefit from access to analyses of the data. Partners are already using clinic data to: assess which pests and crops are frequently brought to the clinics (Jayasundara et al. 2016); prioritise topics for development of new Pest Management Decision Guides; monitor the quality of diagnoses and advice (Danielsen et al. 2013); determine if gender affects the practicality of advice (Lamontagne-Godwin et al. 2016); assess which pesticides are being recommended and provide further training to reduce hazardous pesticide use; and monitor how male and female farmers are being reached through the plant clinics (Alokit et al. 2014).

While data sharing via the POMS requires a username and password, country partners also share data in open forums amongst groups of plant doctors, to monitor clinic activities and validate diagnoses and recommendations. Plantwise is providing forums for discussions on data sharing and use, aiming to facilitate interviews and group discussions, focused on enabling stakeholders to identify opportunities for sharing plant clinic data. Through its work with the Bill and Melinda Gates Foundation (BMGF), CABI has developed tools and models that are being used to map the data ecosystems of target countries, to identify challenges to and incentives for sharing. In each case we recommend next steps and draft national roadmaps for sharing of clinic data with defined audiences.

A number of challenges in managing the data before it can be used effectively were documented by Finegold et al. (2014). After trialling end-to-end data management in a number of countries, it was found that many of the logistic and quality challenges might be overcome by the introduction of ICT to the recording process. This led to piloting tablets in Kenya by plant doctors for recording data and communicating with others in the plant health system (Wright et al. 2016).

Data quality presents a challenge to sharing and using information, and local partners are encouraged to carry out monitoring and quality assurance of the plant clinic data. Data validation includes assessing the quality of a diagnosis and recommendation based on the description captured by the plant doctor on the prescription form (Danielsen et al. 2013). This process is very time consuming, yet it provides valuable insights into the diagnostic strengths and weaknesses of extension staff. An offline tool for validating plant clinic data, used by National Data Validation teams, and data visualisation tools for validated data, are available in the POMS. CABI, through the Plantwise programme, is exploring options to build tools to automate validation, for example to trigger feedback to a plant doctor based on the diagnosis or recommendation that was made.

Responding to Changing Plant Health Problems

Plantwise is committed to the development of tools and processes for assisting rapid response to changing plant health problems encountered by smallholder farmers. A checklist of how to respond to emerging pests was developed for the Plantwise programme and is also used in the Action on Invasives programme, led by CABI.

A number of activities that are already part of the programme can be put into action rapidly in response to emerging threats. For example, a response within 1–2 days should comprise:

- writing an article for the Plantwise Blog based on news from websites, to include relevant information from the Plantwise Knowledge Bank, for example a PDF pack of factsheets about the pest;
- notifying the relevant people and sending them information from the Plantwise Knowledge Bank; for example, contacting CABI Country Coordinators in affected countries to let them know what information is available on a pest outbreak;
- ensuring that there are images in CABI's image management system, tagged so they appear on the Plantwise Knowledge Bank.

There are other activities that should take place within 1–2 weeks:

- finding useful and informative external factsheets by checking known sources and searching on the internet; if a useful factsheet is found it can be indexed as a priority for loading into the Plantwise Knowledge Bank;
- alerting the affected countries about the pest through Telegram; informing them how to recognise it and any advice on management; making sure this message has been validated by CABI scientists before sending if it is not clear what the advice should be;
- extracting POMS records that *might* be the pest; having images submitted with the clinic records reviewed by CABI scientists to see if they might be of the same species so that national partners can be alerted to investigate further;

- updating distribution data for all online CABI products; ensuring that the National Plant Protection Organisation (NPPO) has signed it off if the pest is new to the country.

There are other activities that should take place within 1–2.5 months:

- ensuring coverage on the Plantwise Knowledge Bank, detailing host plant affected, symptoms, prevention and management information;
- creating global crop-specific Green Lists, predecessors to Pest Management Decision Guides, to enable quick dissemination of information as a basis for developing more detailed factsheets;
- commissioning Pest Management Decision Guides and/or Factsheets for Farmers in the countries affected, compiled by specialists and translated into local languages.

Social Media Platforms

The plant clinic network can benefit from development of social network groups on messenger apps (e.g. Telegram, WhatsApp, Line, Viber), which allows plant doctors to share problems amongst themselves and with local diagnostic experts.

Originally, plant doctors trained to use the DCA were set up in groups on Telegram, to encourage sustainability within the programme, and to provide back-up for new users. This social media platform facilitated rapid exchange of information, not only for troubleshooting but also for sharing experience of pest outbreaks and management options (Thakur et al. 2018). Telegram has been used to share policy documents, agronomic resources, meteorological data and even to run lectures on plant health. Members of the Telegram groups reference Plantwise content, and also offer innovative management techniques using household items. Diagnosis may also be facilitated by sharing images of damaged plants with network members, and potentially with CABI's Diagnostic and Advisory Service staff who provide a diagnostic service for fungal and bacterial pathogens, nematodes, viruses, phytoplasmas and insects.

The detection of maize lethal necrosis disease (MLND) in Uganda illustrates how such techniques can be leveraged to quickly combat pest invasions. ICTs were used to initiate a rapid response to an issue in the Tororo district. An Agricultural Extension Officer, unable to diagnose the problem in a maize crop, shared images with the District Agricultural Officer the next day. After a telephone conversation, the Uganda District Agricultural Officer, who had attended a MLND sensitisation workshop held by the National Agricultural Research Organization and the Ministry of Agriculture, diagnosed MLND. It took only 3 days for the Agricultural Extension Officer to return to the farmer with management options. This is in direct contrast to events in Busia district where a more traditional approach of report writing and field visits culminated in a delay of 2 weeks between plant sample collection and confirmation that the problem was MLND (Bundi et al. unpublished).

During the first pilot of ICTs in Plantwise, Kenya was battling with the tomato leafminer, *Tuta absoluta*. Data on this invasive insect was available in the POMS, 65 days earlier when collected on tablets rather than through paper documents. Plant doctors were seen to give higher quality recommendations using the dynamic materials on their tablets, through the Plantwise Factsheets Library app (Wright et al. 2016).

Collaboration with Other Digital Initiatives

Plantwise encourages collaboration with other programmes concerned with global food security. For example, in collaboration with Pestpoint (www.pestpoint.org.au), a digital platform offering advice from peers and experts on evidence-based pest identification, plant doctors in Myanmar, Vietnam and Thailand were provided with a tablet giving access to plant health information, with a magnifier that presented enlarged images of pest problems. The images were reported to facilitate detection and identification of a pest, with the opportunity to share on the Pestpoint platform and seek advice. An external study of the impact of plant clinic data management in the Plantwise programme reported that plant doctors involved in both programmes made use of the Pestpoint tools to support Plantwise-related activities and considered them complementary to their overall work as Agricultural Extension Officers (Sluijs and Posthumus 2017).

The International Food Policy Research Institute (IFPRI) has collaborated with Plantwise in scaling-up the "Seeing is believing" project activities under a CGIAR (Consortium for International Agricultural Research Centers) BigData Inspire Challenge grant. The project aims to help farmers in Tamil Nadu, India, using photographs taken with their own smartphones, to optimise agronomic decision-making for their crops. The project uses Plantwise plant doctors, plant clinics and partnerships for testing research interventions. A plant doctor's role is to promote the importance of picture-based advisories among farmers and to train them in taking photographs using IFPRI's WheatCam app. The Plantwise Knowledge Bank is used as a resource to create pest-based advice (McDade 2019).

PEAT, a German-based company, has developed an app, Plantix (https://plantix.net/en/), for identifying and managing plant health problems in the field. The app includes a community feature in which agricultural stakeholders can share knowledge. Plantix has collaborated with Plantwise in India to evaluate and improve the detection rate of Plantix, with emphasis on control options focussed on the principles of IPM, including biological-based pest management. The pilot uses a network of plant doctors to evaluate and improve the Plantix app. The app uses the smartphone's camera in delivering diagnosis of pests, diseases and nutrient deficiencies based on image recognition. Working as part of the wider Plantwise plant clinic network, and using data gained from the Plantwise Knowledge Bank, Plantix serves as a valuable tool in offering effective pest management advice.

Conclusions

The title of this chapter is "Plantwise: a knowledge and intelligence tool for food security through crop protection". We have shown how Plantwise is both (1) a *knowledge* tool, that can *extend* guidance to farmers to help them manage pests and diseases on their crops, and (2) an *intelligence tool*, that can *gather* local news about those that are currently causing concern. This two-way flow of data – a distinguishing characteristic of Plantwise – is enabled through its deployment by *plant doctors*, especially in the context of *plant clinics*. A key element of such a tool is a dynamic, *authoritative database*.

These characteristics of Plantwise reflect those of the programme's leader, CABI – an international, not-for-profit organization that provides information and applies scientific expertise to solve problems in agriculture and the environment. By sharing science-based knowledge about crop health, through Plantwise and other projects, CABI helps smallholder farmers to grow more and lose less of what they produce, increase their incomes and improve their livelihoods.

As Plantwise operations have progressed since the programme's inception in 2011, new technology has transformed the way pests are reported and managed. Recent projects to develop and future-proof the open and closed platforms at the heart of the programme – the Plantwise Knowledge Bank and the Plantwise Online Management System – have focussed on mobile responsiveness, reflecting the huge increase in access to mobile devices. Emerging activities on social networks, supporting identification, monitoring and management of pests, are just one example of how technology has facilitated rapid information exchange.

Despite advances in technology, the implementation of Plantwise since the first pilot in 2014 has faced many challenges. These include limited digital literacy of plant doctors and farmers, poor enabling infrastructure in the countries, poor network connectivity, and insufficient budgets for providing the required infrastructure. During initial pilots, Plantwise contributed major funds towards creating an enabling environment, through distribution of tablets, internet access, and capacity building for plant doctors (Plantwise 2017). Varying levels of technical skills amongst plant doctors proved to be a major constraint (Wright et al. 2016). A case study of the DCA for plant health in Kenya showed that older plant doctors submitted more plant clinic records than their younger colleagues; and female plant doctors submitted more records than their male counterparts. Generally, plant doctors with higher standards of education, those with more experience, living in urban areas, and using Samsung tablets submitted more records than their less educated, less experienced counterparts, living in rural areas and using Lenovo tablets (Ochilo et al. 2019). Farmers without devices to receive SMS messages, together with poor literacy levels, also present major challenges to providing clinic services electronically. When farmers have phones, the devices are sometimes very basic and either the preferred language is not supported or the limit of the number of characters sent

in one message is low. The handling of local languages during the digitization of data management in Plantwise has also been challenging (Wright et al. 2016; Thakur et al. 2016).

Over time, many countries have invested in purchasing their own tablets, and in some countries plant doctors are willing to use their personal devices. In most cases, Plantwise no longer provides airtime and in general, partners are willing to contribute to the sustainability of e-plant clinics because they see the benefits of ICTs and are keen to switch their clinic operations over to digital (Plantwise 2017).

Plantwise continues to foster new and existing collaborations to ensure the sustainability of the programme in countries where activities have progressed and adapted to opportunities to harness innovation and to promote streamlining and longevity of Plantwise activities. The increasing body of data lends itself to opportunities arising from CABI's role in hosting the Secretariat for the Global Open Data for Agriculture and Nutrition (GODAN) initiative from 2015 to 2019, and the process of linking data sets and working with 'big data'. GODAN is a call to action for better data to drive innovation and economic growth and to feed a growing global population. CABI is preparing to become a leading voice in the global Open Data movement and to catalyse innovative uses of data, for example in the Pest Risk Information Service (PRISE) project. PRISE, the largest technical project to emerge from Plantwise, was launched in 2016 and is funded by the UK Space Agency, linked to the Plantwise programme, and involves a consortium of partners from the UK and countries in sub-Saharan Africa. The project combines novel earth observation technology, satellite positioning, plant health modelling and on-the-ground real-time observations to deliver a science-based information service to predict pest outbreaks.

The implementation of Plantwise has developed into a fusion of ICT and non-ICT approaches, working closely with country partners to maintain information flow and address food security issues of smallholder farmers, using the most suitable delivery techniques for country-specific contexts. New opportunities are focussed on enhancing pest monitoring, reporting and management, together with recognition of lessons learnt in implementing a complex programme spanning many developing countries and opportunities for advancement in new technologies.

Acknowledgements This Open Access book chapter has been made possible within the Plantwise programme, supported by contributions from the UK Foreign, Commonwealth and Development Office (FCDO), the Swiss Agency for Development and Cooperation (SDC), the Directorate General for International Cooperation (DGIS, Netherlands), the International Fund for Agricultural Development (IFAD), the Australian Centre for International Agricultural Research (ACIAR), and the Ministry of Agriculture and Rural Affairs of the People's Republic of China.

References

Alokit, C., Tukahirwa, B., Oruka, D., Okotel, M., Bukenya, C., & Mulema, J. (2014). Reaching out to farmers with plant health clinics in Uganda. *Uganda Journal of Agricultural Sciences, 15*(1), 15–26.

Anderson, J. R., & Feder, G. (2007). Agricultural extension. In R. Evenson & P. Pingali (Eds.), *Handbook of Agricultural Economics* (Vol. 3, pp. 2343–2378).

Bell, M. (2015). *ICT – Powering behavior change for a brighter agricultural future*. Washington, DC: USAID/modernizing extension and advisory services (MEAS).

Bentley, J. W. (2009). Plant health clinics in Bolivia 2000-2009: Operations and preliminary results. *Food Security, 1*(3), 371–386.

Bentley, J., & Boa, E. (2013). The snowman outline: Fact sheets by extensionists for farmers. *Development in Practice, 23*(3), 440–448.

Boa, E. (2009). How the global plant clinic began. *Outlooks on pest management., 20*(3), 112–116.

Bundi, M., Oronje, M. L., Romney, D., & Hunt, S. (unpublished). *Innovative use of ICT in early detection and rapid response of crop pests as a component of advisory service delivery*.

Chernoh, E., & Kuhlmann, U. (2015). The IOBC green and yellow list approach goes global with Plantwise. *IOBC-Global Newsletter, 98*(6).

Chundi, S. (2014). Driving effectiveness of last mile delivery in extension. *Indian Journal of Fertilizers, 10*(9), 104–107.

Connolly, T. M., Boyle, E. A., MacArthur, E., Hainey, T., & Boyle, J. M. (2012). A systematic literature review of the empirical evidence on computer games and serious games. *Computers & Education, 59*(2), 661–686.

Danielsen, S., Boa, E., Mafabi, M., Mutebi, E., Reeder, R., Kabeere, F., & Karyeija, R. (2013). Using plant clinic registers to assess the quality of diagnoses and advice given to farmers: A case study from Uganda. *The Journal of Agricultural Education and Extension, 19*(2), 183–201. https://doi.org/10.1080/1389224X.2012.741528.

FAO. (2013). *Glossary of phytosanitary terms* (International standard for phytosanitary measures 5). Rome: FAO.

Finegold, C., Oronje, M., Leach, M. C., Karanja, T., Chege, F., & Hobbs, S. L. A. (2014). Plantwise Knowledge Bank: Building sustainable data and information processes to support plant clinics in Kenya. *Agricultural Information Worldwide, 6*, 96–101.

International Fund for Agricultural Development. (2013). *Smallholders, food security and the environment*. Rome: United National Environment Programme.

Jayasundara, M. U. P., Herath, Y. M. C. K., Abeykoon, A. N., & Cameron, K. (2016). *The "Permanent Crop Clinic Programme" in Sri Lanka: making use of data to resolve crop health problems*. Poster presented at Annual Symposium of Department of Agriculture 2016, Sri Lanka.

Jenner, W., Cameron, K., Reeder, R. (2020). Emerging Plant Diseases and Global Food Security. Section III: Dtection, Modeling and Evolution. Chapter 10: Plantwise: Monitoring PLant Pest Outbreaks Globally, 201–220. The American Phytopathological Society.

Katherine, H. C., Somachandra, K. P., Curry, C. N., Jenner, W. H., & Hobbs, S. L. A. (2016). Delivering actionable plant health knowledge to smallholder farmers through the Plantwise program. *Journal of Agricultural & Food Information, 17*(4), 212–229.

Lamontagne-Godwin, J., Williams, F., Bandara, W. M. P. T., & Appriah-Kubi, Z. (2016). Quality of extension advice: A gendered case study from Ghana and Sri Lanka. *Journal of Agricultural Education and Extension*. https://doi.org/10.1080/1389224X.2016.1230069.

Leach, M. C., & Hobbs, S. (2013). Plantwise knowledge bank: Delivering plant health information to developing country users. *Learned Publishing, 26*(3), 180–185. https://doi.org/10.1087/20130305.

Masuki, K. F. G., Mowo, G. J., Kmugisha, R. N., Opondo, C. J., & Tanui, J. K. (2011). Improving smallholder farmers' access to information for enhanced decision making in natural resource management: Experiences from southwestern Uganda. In A. Bationo, B. Waswa, J. M. Okeyo, F. Maina, & J. M. Kihara (Eds.), *Innovations as key to the Green Revolution in Africa: Exploring the scientific facts* (pp. 1145–1160). Dordrecht: Springer. https://doi.org/10.1007/978-90-481-2543-2_117.

McDade, M. (2019). Retrieved from https://bigdata.cgiar.org/blog-post/seeing-really-is-believing-when-farmers-photos-revolutionize-insurance-and-advisory-services/

Ochilo, W. N., et al. (2019). Can you ensure that ICT for development apps are downloaded and used? A case study of the plantwise data collection app for plant health in Kenya. *Journal of Agricultural and Food Information*. https://doi.org/10.1080/10496505.2019.1609967.

Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science, 144*(1), 31–43.

Pestpoint website: https://www.pestpoint.org.au. Accessed 26 Sept 2019.

Plantix website: https://plantix.net/en/. Accessed 26 Sept 2019.

Plantwise website: https://www.plantwise.org/. Accessed 30 Sept 2019.

Plantwise. (2017). *Plantwise annual report*. Retrieved from https://www.plantwise.org/wp-content/uploads/sites/4/2019/03/Plantwise-Annual-Report-2017.pdf

Saravanan, R., Sulaiman, R. V., Davis, K., & Suchiradipta, B. (2015). *Navigating ICTs for extension and advisory services. Note 11. GFRAS good practice notes for extension and advisory services*. Lindau: GFRAS.

Savary, S., Willocquet, L., Pethybridge, S. J., et al. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology and Evolution, 3*, 430–439. https://doi.org/10.1038/s41559-018-0793-y.

Sluijs, J., & Posthumus, H. (2017). *An assessment of the use, management and functioning of the Myanmar Plantwise Data Management System. Plant clinic data management Report.* Retrieved from: https://www.plantwise.org/wp-content/uploads/sites/4/2019/03/Sluijs-And-Posthumus-Data-Management-System-Myanmar-2017.pdf

Sones, K. R., Oduor, G. I., Watiti, J. W., & Romney, D. (2015). Communicating with smallholder farming families-a review with a focus on agro-dealers and youth as intermediaries in sub-Saharan Africa. *CAB Reviews, 10*(30), 1–6.

Sustainable Development Goals. (2017). Available online: https://sustainabledevelopment.un.org/sdgs. Accessed 27 Sept 2019.

Tambo, J. A., Aliamo, C., Davis, T., Mugambi, I., Romney, D., Onyango, D. O., Kansiime, M., Alokit, C., & Byantwale, S. T. (2019). The impact of ICT-enables extension campaign on farmers' knowledge and management of fall armyworm in Uganda. *PLoS One*. https://doi.org/10.1371/journal.pone.0220844.

Taylor, P. (Ed.). (2015). *Plantwise diagnostic field guide*. Wallingford: CABI.

Thakur, M., Pandit, V., Chaudhary, M., & Rajikumar, R. (2016). ICT interventions in crop health knowledge management for smallholder farmers. *Journal of Global Communication, 9*, 35–46.

Thakur, M., Pandit, V., Rehman, A., Cameron, K. H., & Beverley, C. (2018). Leveraging information and communication technologies for strengthening plant health extension services in South Asia. In L. N. A. Chandana Jayawardena, M. Suryamani, & C. Sivayoganathan (Eds) *Extnicon 2018: Transforming agricultural extension systems: Towards achieving the relevant Sustainable Development Goals (SDGs) for Global Impact*, 10–12 May 2018, Kandy, Sri Lanka, Sri Lanka Agricultural Extension Association, Sri Lanka, pp. 201–208.

Thompson, M. F., Lyons, A., Kumarasinghe, L., Peck, D. R., Kong, G., Shattuck, S., & La Salle, J. (2011). Remote microscopy: A success story in Australian and New Zealand plant biosecurity. *Australian Journal of Entomology, 50*, 1–6.

Thompson, M., Taylor, P., Reeder, R., Kuhlmann, U., Nolan, C., Mason, J., & Hall, J. (2016). Exploring the value of simulations in plant health in the developing world. In T. Marsh, M. Ma,

M. F. Oliveira, J. B. Baalsrud Hauge, & S. Göbel (Eds.), *Serious games. Proceedings, second joint international conference, JCSG 2016, Brisbane, QLD, Australia, September 26–27, 2016* (Lecture notes in computer science 9894) (pp. 153–162). Cham: Springer.

Toepfer, S., Kuhlmann, U., Kansiime, M., Onyango, D. O., Davis, T., Cameron, K., & Day, R. (2019). Communication, information sharing, and advisory services to raise awareness for fall armyworm detection and area wide management by farmers. *Journal of Plant Diseases and Protection, 126*, 103–106.

Vlachopoulos, D., & Makri, A. (2017). The effect of games and simulations on higher education: A systematic literature review. *International Journal of Educational Technology in Higher Education, 14*, 1–33.

Wright, H. J., Ochilo, W., Pearson, A., Finegold, C., Oronje, M., Wanjohi, J., Kamau, R., Holmes, T., & Rumsey, A. (2016). Using ICT to strengthen agricultural extension systems for plant health. *Journal of Agricultural & Food Information*. https://doi.org/10.1080/10496505.2015.1120214.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

Part V
Plant Diseases and Food Safety

Chapter 12
Pesticide Residues in Food: A Never-Ending Challenge

Carmen Tiu

Abstract Pesticides have been used for centuries to control crop pests and diseases. They are an important tool in integrated pest management. Alongside the pressing demand for food security from a growing population, and with increased international trade in food, there is a corresponding need for greater scrutiny of food safety. This requires improved standards and greater international harmonization in the measurement of pesticide residues in food. There is an urgent need for more complete and rigorous use of Maximum Residue Limit (MRL) standards for pesticides in food. Through an internationally agreed process, MRLs are established for each pesticide intended for use on a specified crop. To provide a realistic contribution to food security and food safety, many more MRLs are needed than are currently available. The chapter examines this urgent practical requirement through a high-level view of the role of pesticides in food security and food safety. A simplified process for establishing MRLs is needed, and an outline scheme is proposed.

Introduction

Pesticides have been used for centuries and their use has increased, in line with the intensity of agriculture. The sustainability of modern agriculture is constrained by many new issues such as limited arable land, changing weather patterns, price volatility reflecting international trade agreements, increasing impact of pathogens, and resistance to pesticides. Tools for managing pathogens and pests according to the principles of integrated pest management (IPM) include cultural practices, pest monitoring, genetic resistance, soil management, pesticides, biologicals, and many more. Every tool can have a driving influence in the management of a given pest, and each is most effective when used in combination with other tools in the toolbox, as applicable from case to case.

C. Tiu (✉)
Global MRL & Import Tolerance Strategy, Corteva Agriscience LLC, Indianapolis, IN, USA
e-mail: carmen.tiu@corteva.com

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_12

Pesticides are a widely used and valuable tool in agricultural production: this chapter aims to present a high-level view of the implications of their use for food security – and especially for food safety, which requires careful regulation, in the context of growing trade in agricultural commodities.

Food Security Versus Food Safety

With the global population growing, and with personal income and acquisition power rising, there has been a significant increase in the demand for food. In addition, the digital era has facilitated communication, and transportation cost has decreased. These changes have resulted in increased trade, so that not only is food more accessible in regions where availability has been limited, but also the demand for ethnically diverse food has increased throughout the world.

Some challenges to illustrate the increased need for food are listed here, based on current statistics from FAO (2018) and WTO (2019).

- The global population will exceed 8 billion by 2030.
- There are currently 1.3 billion people working in agriculture.
- Farmers will have to produce 70% more food to feed the future population.
- Demand for calories is expected to increase by 50%.
- Only 5–10% more land can be used for agricultural production.
- Arable land is not uniformly distributed in the world. Countries with similar arable areas, like India and the USA, may have significantly different production patterns. For example, India employs 98 times more farm workers; yields of cereals are three times greater in the USA.
- Increased pesticide resistance has been amply reported, with examples showing the critical need for rotation of pest control options, and for new options to be constantly developed.
- Fewer new pest control solutions are being brought to the market over time.

In parallel with this increasing quantitative demand for food, there is a growing requirement for greater scrutiny of food quality, and especially food safety. More stringent food regulation is called for, alongside restrictions applied by the food value chain (food industry, consumers, non-government organizations, etc.) and through self-regulation (e.g. private, or self-certification networks). A few factors that illustrate the current status of global change are listed here, based on data from the United Nations Statistical Department (UNDESA 2019).

- Trade of fruits and vegetables increased in value and amount 2700 times during the first decade of the new millennium (2000–2010).
- Increased scrutiny for food residues (e.g. USA, Canada, Australia, EU, Japan, Korea, Taiwan, and many others).
- Changing thresholds for monitoring. Many countries have regulations defining anything below the analytical method's level of discrimination (typically

0.01 mg/kg, except Canada and Australia having 0.1 mg/kg); traces are usually acceptable because they add a negligible amount to the consumer's intake and dietary risk.

- Increased regulation for residues, metabolism, toxicity data of parent molecules, metabolites and breakdown compounds.
  - Number of studies required for registration and re-registration.
  - More countries have new or increased regulations.
  - Additional safety factors incorporated in risk assessments.
  - Lack of harmonization of requirements and their acceptance criteria.
  - Secondary (non-regulatory) restrictions (e.g. half, or one-third of MRL, no residues, no more than two pesticides, etc).

Pesticide residue limits are of special importance in this context. Despite the potential complexity of their assessment and in the application of standards, this basic principle is simple, and has been in place since 1985, in the FAO *Submission on Pesticide Residues*, now in its third edition (FAO 2016):

> Good agricultural practice in the use of pesticides (GAP) includes the nationally authorized safe uses of pesticides under actual conditions necessary for effective pest control. It encompasses a range of levels of pesticide applications up to the highest authorized use, applied in a manner which leaves a residue which is the smallest amount practicable. Authorized safe uses are determined at the national level and include nationally registered or recommended uses, which take into account public and occupational health and environmental safety considerations.

Similar considerations on the correct use of pesticides are included in the FAO International Code of Conduct on Pesticide Management, 2014 http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/code/en/.

Trade Impact on Food Regulation

According to FAO statistics, the value of food trade in 2013 was USD 1.12 trillion, and has been steadily growing with an exponential trend after the year 2000. There have been many initiatives to establish best management practices and global harmonization to enable food security through trade, while keeping and improving food safety.

On 7–8 April 2016, the Meeting of Agriculture Ministers of the Organisation for Economic Co-operation and Development (OECD) brought together 46 countries, plus European Union Member States, and produced a *Declaration on Better Policies to Achieve a Productive, Sustainable and Resilient Global Food System.* This *OECD Declaration* presents some important principles for establishing uniform national policies, for example:

- Be consistent with economy-wide measures, in relation to growth, development, trade, investment, employment, well-being, and the environment.

- Be transparent, targeted, tailored, flexible, consistent and equitable, while ensuring value for money for scarce government resources.
- Support better multilateral trade systems to enable opportunities for competition among suppliers, on an equitable, transparent, non-discriminatory basis.
- Make innovation a priority in order to achieve sustainable productivity growth.
- Foster production systems that use available water, land, forest, energy, soil and biodiversity resources sustainably; promote animal, plant and human health.
- Foster resilience of farmers to risk, to enable them to handle more frequent unpredictable events, such as weather-related shocks, disease outbreaks, and market volatility.

Many countries have adopted principles like these, through bilateral and multilateral Free Trade Agreements (FTA) and Ministerial Agreements (MA). An example is the initiative led by the USA, Kenya and Uganda, which delivered a document signed by Agricultural Ministries from 17 countries, on December 12, 2017, at the World Trade Organization (WTO) in Geneva. This MA document aims to provide trade-based solutions to non-tariff barriers, for example through harmonization of existing tolerances of Maximum Residue Limit, and through provision of MRL standards, for the far more numerous cases where they are missing.

The *Agreement on the Application of Sanitary and Phytosanitary Measures* (the "SPS Agreement") entered into force with the establishment of the WTO, on 1 January 1995. It concerns the application of food safety, and animal and plant health regulations. The SPS Agreement states that "to harmonize sanitary and phytosanitary measures on as wide a basis as possible, Members shall base their measures on international standards, guidelines or recommendations". The Agreement names the joint FAO/WHO Codex Alimentarius as the relevant standard-setting organization for food safety. Since the 1960s the Codex has developed over 200 standards covering raw, semi-processed or processed foods intended for consumption, or for intermediate processing; established over 40 hygienic and technological codes of practice; evaluated over 1000 food additives and 54 veterinary drugs; set more than 5000 MRLs, for about 250 pesticide residues; and specified over 30 guidelines for contaminants.

In this context, multiple stakeholders are involved in following regulations for trade standards, representing different segments of the food value chain (Table 12.1).

Communication and coordination amongst stakeholders is critical to enable the success of future regulatory updates and their consistent implementation across countries, commodities, and related food value chain processes, from the farm to the dinner table.

Table 12.1 Categories of stakeholders and their activities as they relate to food standards

AGENCIES	Harmonization of guidelines (OECD)
	Harmonization of acceptance criteria (Codex Alimentarius)
	Harmonization of global regulatory compliance (SPS Agreement)
REGISTRANTS	Safer products to people and environment
	Application by digital precision technologies
	Globally harmonized programs
PRODUCERS	Responsible use, integrated multiple tools
	Compliance of multiple standards
PROCESSORS	Private compliance programs
	Global unified production standards
CONSUMERS	Increased knowledge by e-tools
	Increased confidence in agencies and weight of evidence

Trends in Food Regulation

The traces that pesticides leave in treated products are called residues. The most important trade standard is the Maximum Residue Limit (MRL); synonyms are Maximum Residue Level and (in the USA) Tolerance. The MRL is the maximum concentration of pesticide residue that is legally permitted, or recognized as acceptable, on an agricultural commodity (such as food), when pesticides are applied correctly – in line with Good Agricultural Practice (GAP), as specified on the product label. Residues in imported food, derived from authorized uses in foreign countries, are also controlled through establishment of Import Tolerances, where no domestic use is foreseen – e.g. for tropical crops that do not grow in temperate countries.

The aim is to set MRL values so that pesticide residues found in food are as low as possible and safe for consumers, after rational use to protect crops. An MRL provides a legal check that commodities have been treated with pesticides according to approved GAP standards, as stated on the product label in terms of: application rate, number of applications, pre-harvest interval (PHI), intervals between application, application method, cultural practices, etc. An MRL is not a safety, or health standard: a risk assessment process is used to review the safety of established, or proposed MRLs (but is not used to calculate the actual limit).

MRLs are also set by agencies from 14 countries or regions, based on field residue trials in supervised crops conducted in compliance of national requirements. These national MRLs (e.g. US-EPA, EU-MRL) as well as Codex MRLs are currently adopted, either by deferral, or by a conversion procedure, in about 70 other countries. In addition, potentially, the entire list of 189 member countries of the Codex Alimentarius are likely to adhere to similar standards. The scientific guidelines for conducting residue trials and setting MRLs have been fairly harmonized by OECD (2019), and these standards are followed to a large extent by all countries setting, or implementing MRLs.

An MRL for a pesticide intended for use, on a specified crop, is established by a sequential process:

1. Crop field trials are conducted according to label/GAP and following OECD Residue Chemistry Guidelines http://www.oecd.org/chemicalsafety/pesticides-biocides/publicationsonpesticideresidues.htm.
2. Residues in food commodities are analyzed at harvest and, if results are quantifiable, residue data after typical processing are also required.
3. Replicated trials are analyzed to account for variability.
4. An MRL value is recommended to account for the range of data and by applying the statistical OECD MRL Calculator http://www.oecd.org/env/mrl-calculator-users-guide-and-white-paper-9789264221567-en.htm.
5. A dietary risk assessment is made to confirm that there will be no risk to consumers, based on consumption patterns from different countries, or on global diets published by WHO https://www.who.int/nutrition/landscape_analysis/nlis_gem_food/en/.
6. MRLs are also periodically re-evaluated for compliance, by the agencies that originally set them. This is typically done by evaluating monitoring results from the national heath surveillance programs. In general, monitoring results across agencies over the past 2–3 decades are showing around 1% of MRL violations and no risks to consumers have been identified for those cases, e.g. European Food Safety Authority (EFSA) yearly reports https://www.efsa.europa.eu/en/efsajournal/pub/5348).

However, although regulations, guidelines, and requirements have been through numerous harmonization initiatives, the currently available MRLs are far from being uniform across national agencies.

There is a clear need for greater international harmonization of MRLs, following the guidance of the FAO International Code of Conduct on Pesticide Management, 2014. This Code indicates that responsibility should be shared between governments and the pesticide industry. Governments should introduce policy and legislation for the regulation of pesticides; should make provision for its effective coordination and enforcement (6.1.1); and should regulate and monitor pesticide residues in food (6.1.14). The pesticide industry should conduct residue trials prior to marketing, in order to provide a basis for establishing appropriate MRLs (4.1.7).

Trends for Food Security and Food Safety

As a basis for food security and food safety, significantly more MRLs are needed, than are currently available. For example, the EU's list of MRLs includes numerous entries set at the limit of analytical quantification (LOQ). Some of these reflect actual estimates of MRL, but the majority are default values; these can be considered as missing MRLs.

The magnitude of this issue is so big that a simpler process is needed for setting MRL values. Ideally, a "ONE-MRL" concept should be adopted, such that the MRL value, set when the first use of a pesticide is authorized on a crop, can be considered as globally acceptable, unless formally challenged. A sequential process for such a concept is outlined here.

1. The national agency to first review a new use sets an MRL and, under the SPS Agreement, must notify all other countries, giving them the opportunity to comment if international trade might be affected.
2. This notification triggers a process at the Codex Committee on Pesticide Residues (CCPR) to conduct a peer-review of the proposed MRL's global appropriateness (the same way it already triggers global notification through the WTO-SPS network).
3. The CCPR also conducts periodic reviews of existing MRLs, on the basis of data provided by member countries, to identify any potential disparities or trade irritant evidence.
4. These steps are repeated at any time in response to a formal challenge of an existing MRL from any member of the Codex Alimentarius Commission.

This type of procedure has already been validated and implemented at regional scales. For example, the USA, Canada and Mexico started a harmonized process for the North American Free Trade Agreement (NAFTA) region in 1999. The EU started a process for harmonized assessment of MRLs in 2006. Australia, the USA and Canada proposed a harmonized guideline for Import Tolerances, within the 21 economies of the Asia and Pacific Economic Cooperation (APEC) in 2016.

Conclusion

This book is about the impact of plant diseases on food security. It is easy to overlook food safety as a component of food security, including the special case of safety in the rational use of pesticides. This chapter addresses that issue, focusing on the never-ending challenge of managing pesticide residues in food. As the global demand for food rises, pesticides will increasingly be needed as a component of integrated pest management. Good agricultural practice requires that they be applied so as to leave the smallest practical residue. To this end, there is an urgent need for more complete and consistent use of MRL standards for pesticide residues in food. This need cannot be met through the currently accepted processes. It is hard to avoid the conclusion that a simplified procedure is needed to increase global efficiency for setting harmonized MRLs. This chapter outlines such a procedure, as an example of how this pressing requirement could be met.

References

FAO. (2016). *Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed* (FAO, plant production and protection paper 225). ISSN 0259-2517. http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Manual/FAO_manual_3rd_edition_Final.pdf

FAO. (2018). *Food and Agriculture Organization of the United Nations, Statistical Division*. http://www.fao.org/statistics/en/

OECD. (2019). *OECD test guidelines for chemicals*. http://www.oecd.org/env/ehs/testing/oecd-guidelinesforthetestingofchemicals.htm

UNDESA. (2019). *United Nations Department of Economic and Social Affairs, Statistics*. https://www.un.org/development/desa/en/key-issues/statistics.html

WTO. (2019). *World Trade Organization, Trade Topics*. https://www.wto.org/english/tratop_e/tratop_e.htm

Chapter 13
How Can Plant Pathology Help in the Control of Human Pathogens Associated with Edible Crop Plants?

Nicola Holden

Abstract Edible plants are an important vehicle in the transmission of human pathogens. Most such pathogens are food-borne, deriving from plants that are eaten raw or minimally processed. Molecular interactions between foodborne pathogens and plant hosts show similarities with those between plant pathogens and their hosts, although important distinctions are also evident. For example there are parallels between PAMP-triggered immunity to human pathogens and elicitation of defence responses in plants to their pathogens. Control of human pathogens on plants is exercised through food safety and risk management. The important overlap between crop protection and management of crop-derived human pathogens opens opportunities for the two disciplines to benefit from shared dialogue and research.

Introduction for Human Pathogens

Produce-Associated Foodborne Outbreaks

Foodborne pathogens are frequently associated with consumption of fresh fruits and vegetables. In some countries, e.g. the USA, plant-based foodstuffs account for more than 50% of foodborne illnesses (Painter et al. 2013). Although it is likely that plant-based foods have always been responsible for a proportion of foodborne illness, it was only after large-scale outbreaks in the mid-2000s that the issue attracted substantial attention, for example after an outbreak of *Escherichia coli* associated with spinach in the USA in 2006 (Centers for Disease Control and Prevention (CDC) 2006). Notable events in 2018 included an outbreak of *E. coli* O157:H7 from consumption of contaminated romaine lettuce, involving 210 reports, 5 fatalities

N. Holden (✉)
Cell and Molecular Sciences, The James Hutton Institute, Dundee, UK

Rural Land Use, SRUC, Aberdeen, UK
e-mail: nicola.holden@sruc.ac.uk

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2_13

and 27 cases of hemolytic uremic syndrome. There were reports of cucumbers contaminated with *Salmonella enterica* serovar Agona, including 147 reported cases of salmonellosis centred in the UK, and others in Finland, Germany and Ireland. There was also a widespread outbreak of the protozoan parasite *Cyclospora cayetanensis* in the USA, with 511 cases arising from contaminated salad sold at fast-food restaurants (Centers for Disease Control and Prevention 2018b) and 250 cases from vegetable trays (Centers for Disease Control and Prevention 2018a).

Causative Organisms

A number of different human pathogens are associated with plant produce, the common factor being transmission through the food chain, either during crop growth or post-harvest. All classes of pathogens have been implicated including viruses, protozoan parasites, bacteria and fungi. Many have an animal source, farmed livestock or humans being the primary reservoir. The pathogens can be split into two groups: those species (mostly bacteria) that actively interact with plants, having the potential to use plants as secondary hosts, and those (mostly viruses and protozoa) that are passively transmitted into the food chain, by plants acting merely as fomites in the transmission pathway. A third group of pathogens, less frequently implicated, are those normally associated with plants, either in a commensal non-pathogenic state or as phytopathogens. This group includes some opportunistic fungal phytopathogens (Dickman and de Figueiredo 2011).

Consideration of the role of plants in the life cycle of human pathogens overlaps with many aspects of plant pathology and plant-microbe interactions. As such, these disciplines can provide valuable insights into mechanisms of colonisation and transmission by human pathogens, and their control. Indeed, examination of the molecular basis of interactions between human pathogens and plants serves to highlight this area as an extension of plant pathology and plant-microbe interactions, rather than as a distinct discipline. Furthermore, the interactions are not limited to plants in their pre-harvest state, but are also valid post-harvest, where the overlaps are with food-spoilage microbes. In this chapter the focus is on bacteria-plant interactions that occur in living, pre-harvest plants, drawing on the extensive body of work within plant pathology.

Foodborne Pathogens

The most frequently reported foodborne bacteria associated with plants are shiga-toxigenic *E. coli* (STEC), non-typhoidal *Salmonella enterica*, and *Listeria monocytogenes* (FAO/WHO 2008). Sub-species variants occur in produce-associated outbreaks, including a number of different *S. enterica* serovars. The most frequent STEC serotype is O157:H7. *L. monocytogenes* is considered to be a soil-associated

pathogen, with quite distinct physiology and life cycle from the Enterobacteriaceae. It is frequently characterised by its ability to grow at low temperatures, and an ability to form recalcitrant biofilms, which means that it is often associated with post-harvest contamination (Freitag et al. 2009). *E. coli* and *S. enterica* belong to the Enterobacteriaceae and show similarities in physiology, metabolic requirements and routes of transmission in the food chain (Neidhardt et al. 1996), and their primary reservoirs are farmed animals. They are genetically related to important plant pathogens of the genus *Erwinia* causing soft-rots. Genomic comparisons highlight the similarities, including potential factors for plant colonisation (Toth et al. 2006; Holden et al. 2009). An important aspect of this family of bacteria is their metabolic flexibility and, as mesophiles, their capacity to proliferate under a wide range of physio-chemical environments, which underpins their ability to grow and persist in an astonishingly wide range of hosts and habitats. In contrast, *L. monocytogenes* has more fastidious requirements, which may give rise to a degree of 'niche exclusion' and may explain the relative prevalence of *E. coli* and *S. enterica* in outbreak reports.

Plant-Microbe Interactions

Localisation of Human Pathogens on Plant Tissues

Foodborne bacteria have been reported to associate with all types of plant tissues. In keeping with localisation of endemic plant-associated microbes, the roots and rhizosphere appear to present a preferred niche (Berg et al. 2005). This ecological habitat is rich in nutrients, and relatively buffered from temperature and humidity fluctuations compared to foliar tissue (Bais et al. 2006; Holden 2018). Indeed, apparent die-off or reduction in viable, culturable cells has frequently been reported for foodborne pathogens associated with foliar tissue, compared with those associated with the roots and rhizosphere (Dong et al. 2003; Kisluk and Yaron 2012; Quilliam et al. 2012).

Genomic Comparisons with Plant-Associated Bacteria

Phylogenomics of human and phytopathogenic bacteria show similar patterns: species within shared taxonomic families show a trend towards clonality or alternatively (but not exclusively) extensive recombination. This is demonstrated by the Enterobacteriaceae, where *Escherichia* and *Pectobacterium* species show similar patterns of mosaicism in their genomes, indicative of divergence and multiple recombination events (Pritchard et al. 2016; Dixit et al. 2017). A similar pattern is seen for the generalist pseudomonads, such as the *Pseudomonas fluorescens* species complex (Scales et al. 2014) and the xanthomonads (Jibrin et al. 2018). On the other

hand, clonality is more evident for some soil-associated bacteria, such as *Listeria* species (Ragon et al. 2008) or specialist plant pathogens such as *Xylella fastidiosa* (Nunney et al. 2014). However, more detailed examination of any species inevitably reveals a degree of clonality (Tibayrenc and Ayala 2012). The differences in recombination and divergence are a reflection of promiscuity for DNA uptake, selective pressures and different physiological responses to stresses. The result is a wide range of genotypes that enable colonisation and persistence on plant hosts. Genomic comparisons between different groups of bacteria have been used to identify potential plant colonisation factors, both of the human pathogen *Klebsiella pneumoniae* (Holden et al. 2009) and the phytopathogen *Pectobacterium atrosepticum* (Toth et al. 2006). However, successful colonisation is likely to be dependent on a combination of factors rather than a single genetic component.

Evolution Towards a Plant-Adapted Lifestyle

Pathogens evolve in response to selective pressure, and there are some indications that foodborne human pathogens have evolved in association with plant hosts. For example, phylogeny of STEC shows distinct clades associated with different plant species, such that serotypes normally associated with cattle, e.g. O157:H7, have also been linked to outbreaks from leafy salads like spinach and lettuce, whereas other serotypes, e.g. O104:H4 and O111, have been linked to sprout- and flour-associated outbreaks (Hao et al. 2012). Whether these differences are due to selective pressure of the plant-environment or to differences in the primary reservoir is not yet clear, but this does raise the possibility of the plant environment as an evolutionary driver. *E. coli* is widespread in nature and prevalent in arable agriculture, although showing wide genetic diversity (Holden et al. 2013). Environmentally persistent *E. coli* are termed ‘naturalised’ and have been identified in water (Walk et al. 2007) and soil (Brennan et al. 2010); they could act as donors for animal-derived isolates via recombination and genetic exchange. Therefore, it is less straightforward to assign pathogenic features to non-pathogenic interactions in one host or another, especially for the generalist bacteria. Retrospective pathogen characterisation can be made only after the advent of symptomatic disease, and this then generally informs on the features relating to (human) pathogenicity. The ability to persist in secondary hosts such as plants must then be defined by experimentation or chance occurrence from surveillance.

Attachment

Attachment to host tissue is considered to be a prerequisite for colonisation. Multiple mechanisms of attachment are employed by bacteria, from non-specific interactions to specific recognition of host-derived targets. Attachment to plant hosts is covered

in more detail elsewhere (Holden et al. 2012), but in general adherence mechanisms are better described for the interactions of human pathogens with animal tissue than for those between plant-associated bacteria and plant tissue, since adherence is considered as a virulence factor for human pathogens, linked to development of infection in humans. Although there are some overlaps between mechanisms of binding between biological kingdoms, they appear to be restricted to non-specific mechanisms of attachment, e.g. via flagella (Rossez et al. 2015). On the other hand, adherence mechanisms based on specific interactions are only shared between biological kingdoms when the target receptors and ligands are shared. An example of this is mannans, presented as polysaccharides, oligosaccharides or glyco-proteins of mannose, which are a target for the Type 1 fimbriae adhesin, FimH, encoded by *E. coli*, *Klebsiella pneumoniae* and *S. enterica*. Although different glycosidic linkages occur for mannans derived from animal and plant kingdoms, both forms can be recognised by *E. coli* FimH, albeit with differences in specificity (Marshall et al. 2016). On the other hand, some adhesins can only function for plant hosts since the target glycans are not present in the animal kingdom; an example is *E. coli* common pilus (ECP), which binds to arabinans (Rossez et al. 2014). This functional specificity is coupled with regulatory control that is appropriate for plant-relevant temperatures. Together, these observations point to a model of adherence for bacteria interacting with plants, starting with a form of non-specific adherence, which may be sufficient for proliferation and development of colonies and biofilms, as seen with soil-associated *Bacillus* species in the rhizosphere (Beauregard et al. 2013). For some species, the initial interaction is then followed by a more robust mechanism of binding, via adhesins, which provides a secure interaction (Rossez et al. 2014), and thus a starting place for colony establishment in the rhizosphere. Attachment to the phyllosphere has not been investigated to the same extent, but the human pathogens also exhibit elicitation of adherence factors to facilitate biofilm formation, such as curli fibres (Wright et al. 2017). Fimbrial attachment for plant pathogens has been described for *Erwinia amylovora* in xylem tissue (Koczan et al. 2011) and unipolar polysaccharide fimbriae have been described for *Agrobacterium tumefaciens* (Fritts et al. 2017)*, although these appear to be involved in biofilm formation rather than attachment to plant surfaces per se.*

Defence Response

Since the general mechanisms of the initial plant-microbe interactions are shared between plant-associated bacteria and human pathogenic bacteria, similarities could be expected in the plant defence response, in particular in the pathogen/microbe-associated molecular pattern (PAMP/MAMP) response. Human pathogens can colonise both foliar and root tissue, and although the plant defence response has been most widely described for leaves, similar responses have been reported for roots e.g. in *Arabidopsis thaliana* (Millet et al. 2010). Key elicitors of PAMP-triggered immunity (PTI) are molecular patterns that are abundant, widespread and

well conserved in microbes. Indeed, they can induce innate immune responses in both animal and plant kingdom eukaryotes, highlighting the shared heritage of basal defence. These include the surface-expressed organelles of flagella (Gomez-Gomez and Boller 2000) and lipopolysaccharide (LPS) (Zeidler et al. 2004), and the cytosolic proteins elongation factor (EF-Tu) (Kunze et al. 2004) and cold shock proteins (CSP) (Felix and Boller 2003). Recognition of the molecular patterns by the host report on either intact bacterial cells or cellular components from lysed/disrupted cells. Recognition occurs via well established pathways, starting with binding of the molecular pattern by the cognate plant pattern recognition receptor (PRR) in concert with cofactor proteins (Segonzac and Zipfel 2011), triggering signalling cascades (Bigeard et al. 2015) and induction of defence responses that strengthen cell walls (Voigt 2014) or produce antimicrobials (Qi et al. 2017). While 'professional' plant pathogens can deliver effector proteins that subvert PTI and hence reduce its impact on microbial clearance by the plant host (Boller and He 2009), whether the same occurs for human pathogens is uncertain.

Activation of PTI by bacterial flagella has been extensively studied and in plants requires binding of a conserved peptide of flagella to the PRR receptor FLS2 in concert with BAK1 (Chinchilla et al. 2007). Flagella derived from human pathogenic bacteria, e.g. *S. enterica*, are also recognised by FLS2 and lead to a defence response in *A. thaliana* (Garcia et al. 2014). However, in an effort to identify bacterially associated PAMP responses in *A. thaliana*, inoculation with a flagellin mutant of *E. coli* O157:H7 did not result in significant PAMP-induced gene expression changes compared to a flagellin wild-type strain (Thilmony et al. 2006). Occurrence of a similar pattern for a pair of *Pseudomonas syringae* flagellin +/− strains leads to the speculation that flagella organelles in the context of bacteria have a minor PTI impact and are only one of several PAMPs responsible for triggering basal immunity. It does appear, however, that the site of inoculation influences the response, perception of flagellin being stronger on leaf surfaces than in the intracellular spaces (Thilmony et al. 2006; Zipfel et al. 2004).

Identification of the CORE receptor for the CSP peptide indicates that there is some taxonomic specificity in the plant response, as CORE only appears to be present (to date) in the family Solanaceae (Wang et al. 2016). However, its expression in *A. thaliana* resulted in a functional immune response and with concomitant reduction of phytopathogens used in a challenge assay. CSP are ubiquitous in bacteria across diverse taxonomic lineages (Fig. 13.1) and normally present in multi-copy. Moreover, they are abundantly expressed at temperatures relevant to colonisation of plant hosts (Phadtare and Inouye 2008). Therefore, it is perhaps unsurprising that they are perceived as PAMPs in plants. It is notable, however that they are not detected in an analogous manner in animal hosts. The same appears true for the PAMP EF-Tu. In animals, perception of PAMPs/MAMPs occurs via Toll-like receptors, which share functional and structural similarities to plant PRR. Animal PAMP perception of LPS and flagellin occurs via TLR-4 and TLR-5, respectively (Takeda et al. 2003). It is possible that differences in the repertoire of cross-kingdom PAMP perception occur as a result of PAMP expression differences in association with the different hosts. For example, five of the *E. coli* O157:H7 *csp* genes were

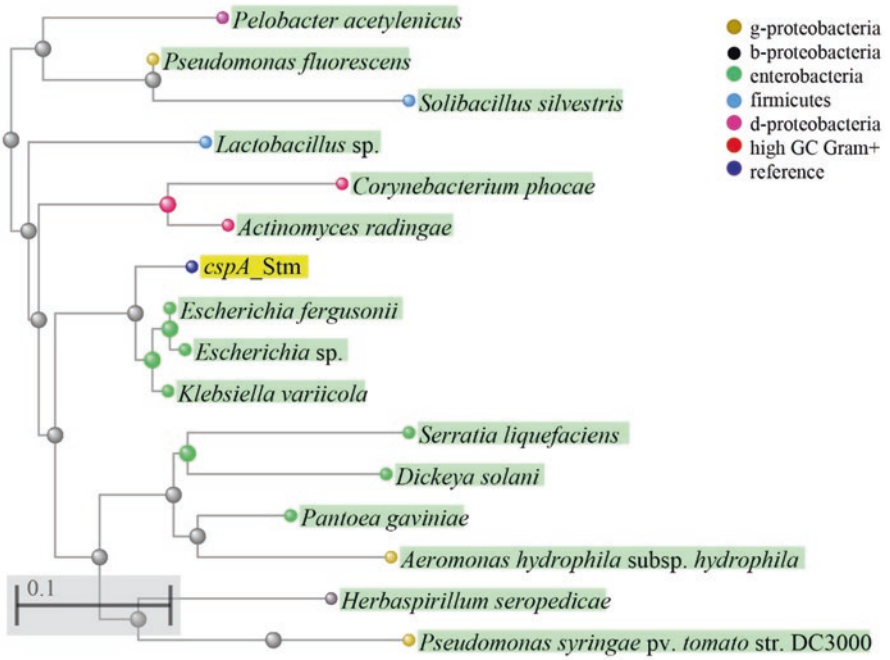

Fig. 13.1 Phylogenetic alignment of *csp* gene sequences. Representative *csp* gene sequences from a sub-set of divergent bacteria (indicated by coloured nodes) were selected by BLASTn alignment to a reference sequence (*cspA*_Stm – highlighted yellow: *cspA* gene from *S.* Typhimurium), and a phylogenetic tree generated using a fast, minimum-evolution algorithm. (Desper and Gascuel 2004)

substantially differentially induced (>nine-fold) on exposure to spinach leaf extracts, while the genes encoding EF-Tu (*tuf*) were also induced (~1.5-fold) (Crozier et al. 2016).

Bacterial effector proteins that supress basal immunity do so by targeting components of the signalling cascade or transcription factors. Since basal immunity in eukaryotes is ancestrally shared, there is potential for cross-kingdom functional activity of bacterial effectors (Brunner and Fraiture 2014; Zipfel and Felix 2005). A clear example was demonstrated for the *S. enterica* serovar Typhimurium effector, SpvC, which functions in animal hosts to suppress basal immunity and was shown to suppress a flagellin-dependent PAMP-response similarly in *A. thaliana* protoplasts (Neumann et al. 2014). Like the *Ps. syringae* effector AvrPto, *S.* Typhimurium SpvC inhibited *FRK1* expression, although its range of inhibition was not as extensive as the phytopathogen effector counterpart. SpvC was shown to act in plant hosts in the same manner as in animal hosts (Mazurkiewicz et al. 2008), by dephosphorylating activated MAP-kinases, a central component of the signalling cascade. Furthermore, its deletion resulted in a decreased ability of *S.* Typhimurium to proliferate in infiltrated *A. thaliana* leaves (Neumann et al. 2014).

The ‘professional’ phytopathogens are well equipped to manipulate immune responses with a suite of effector proteins, inducing effector-triggered immunity (Jones and Dangl 2006). Specificity in the interaction for this group of pathogens results in differences in the outcome for compatible host and non-host interactions (Senthil-Kumar and Mysore 2013). Phytopathogen effectors are secreted via a type-3 secretion system (T3SS) (Grant et al. 2006), and although human pathogens similarly secrete their effectors via the T3SS in animal hosts there are structural and potential regulatory differences between the two groups of bacteria (Roe et al. 2003). This raises the question about the potential cross-kingdom scope of human pathogen-derived effectors, and how they are expressed in plant hosts. It thus appears that the extent of plant immunity in response to human pathogens occurs at the PAMP-level. This is borne out by the observation for *E. coli* O157:H7 that induction of the PAMP response in infiltrated *A. thaliana* leaves overlapped with a non-pathogenic *Ps. syringae* strain lacking a T3SS (*hrp*-) (Thilmony et al. 2006). As such, human pathogens lend themselves to more detailed investigation of innate immunity in plants, without the masking effect of Effector-Triggered Immunity (ETI).

Establishment and Metabolism

Following initial attachment and successful avoidance or countering of the host defence, bacterial colonies become established. The patterns of colonisation of human pathogens on plants bear some similarities with those of plant-associated bacteria, e.g. between *E. coli* O157:H7 and *P. atrosepticum* (Wright et al. 2013). Differences are also evident, for example *E. coli* O157:H7 on spinach roots accumulates on the surface and in natural crevices, i.e. between cells or surrounding the base of root hairs, while *P. atrosepticum* on potato roots tends to invade root epidermal cells, with less density of bacteria evident on the cell surfaces (Fig. 13.2). This is presumably a result of the invasive phenotype conferred by the activity of plant-cell-wall-degrading enzymes in *P. atrosepticum* (Toth et al. 2006). *E. coli* O157:H7 also has the ability to internalise without the benefit of extracellular enzymes. It has been found to enter plant cells, and can be present in the extracellular apoplast space in roots (Wright et al. 2013) and leafy tissue (Wright et al. 2017). The basis for its entry into plant cells is unknown but could be related to exploitation of senescent cells. On the other hand, the ability to enter into the apoplast of either root or foliar tissue is widely reported for human pathogenic bacteria, although mostly demonstrated under laboratory conditions (Deering et al. 2012; Hirneisen et al. 2012); it appears that human pathogens, like plant-associated bacteria can take advantage of natural openings such as stomatal pores (Wright and Holden 2018) or emergence of lateral roots (Wright et al. 2013).

Successful establishment and development of colonies on plant host tissue requires appropriate metabolism. The role of metabolism as a central facet of adaptation to host or habitat is well accepted and is covered in more detail elsewhere for human pathogens (Alteri and Mobley 2012; Rohmer et al. 2011) and for

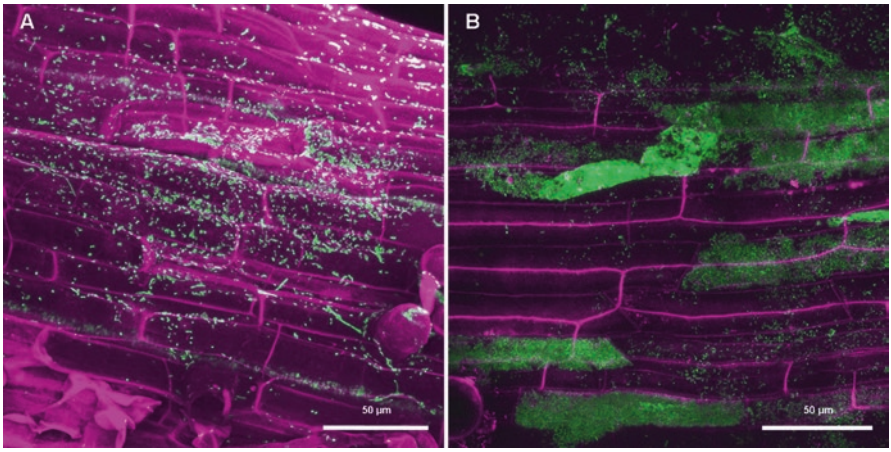

Fig. 13.2 Patterns of root colonisation by human pathogenic or phyto-pathogenic bacteria. Spinach plants inoculated with *E. coli* O157:H7 (isolate Sakai) (**a**) and potato plants inoculated with *P. atrospecticum* (isolate SCRI-1043) (**b**) were collected 6 days after inoculation, and the roots imaged by laser scanning con-focal microscopy. Plant tissue was labelled with Texas red to stain the cell walls (magenta) and both bacterial isolates are expressing plasmid-borne GFP markers (green). The experimental set-up was part of the study described previously. (Wright et al. 2013)

plant-associated bacteria (Holden 2018; Hugouvieux-Cotte-Pattat 2016; Lodwig and Poole 2003; Price-Whelan et al. 2006). Substantial transcriptional changes occur for human pathogens on contact with plant tissue or plant-derived substrates (Goudeau et al. 2013; Kyle et al. 2010; Mark et al. 2005), with specificity for different plant tissues from the same species, e.g. differential responses of *E. coli* O157:H7 to root exudates, leafy lysates or leaf cell wall polysaccharides of spinach (Crozier et al. 2016). Such differences in metabolic pathways can be mapped and linked to available substrates (Holden 2018) and, for the pseudomonads, this has been shown to be associated with a generalist or specialist life-style on plant hosts (Mithani et al. 2011). Thus, for human pathogens, much like their plant-associated counterparts, metabolic flexibility and capacity is a key factor in successful colonisation of plant hosts.

Control and Management Approaches

Transmission Pathways

The primary reservoir for most foodborne human pathogens is farmed animals, while some, such as *Listeria monocytogenes*, are considered to be soil saprophytes. Regardless of primary source the main transmission routes onto plants during the pre-harvest stage are from contaminated soil or contaminated irrigation water.

Irrigation water is seen as the most important route of transmission (Allende and Monaghan 2015). Plants grown outdoors are subject to other inputs, e.g. direct manure contamination, prior contamination of the soil from manure, or contamination from wild animals or birds, as was the case for the large-scale outbreak of *E. coli* O157:H7 from spinach plants in 2006 (Jay et al. 2007). Another potential source is from contaminated seeds, which may have accounted for the large-scale outbreak of *E. coli* O104:H4 from fenugreek in 2011 (Buchholz et al. 2011). This isolate and other human pathogens have been shown to persist for prolonged periods on seeds (Knodler et al. 2016; Van der Linden et al. 2013), and analysis of endemic seed microbiomes shows the potential for this route of transmission of human pathogens (Nelson 2018).

Geographical Differences

Most of the causative organisms of foodborne illnesses are reportable in wealthy countries. Data from this source, and from epidemiological questionnaires for food source attribution, have resulted in detailed databases that allow rational implementation of control measures. A clear example of this arose out of the very large-scale outbreak of *E. coli* O104:H4 from fenugreek in the European Union (EU), in 2011 (Buchholz et al. 2011), with implementation of EU legislation for microbiological criteria ((EU) No 209/2013) and traceability ((EU) No 208/2013) of sprouted seeds. Many countries do not have the infrastructure or systems for reporting foodborne illness in such detail, and this has resulted in a somewhat skewed picture of where the problem occurs. However, given that the main routes of transmission are from irrigation water (Allende and Monaghan 2015) and animal manure (FSA 2009), the burden of disease from consumption of contaminated fresh produce crops is likely to be equally high or even higher in these countries. Furthermore, diarrhoeal disease is one of the major causes of ill health worldwide, with an estimated 600 million (almost 1 in 10 people) worldwide falling ill after eating contaminated food (WHO 2018), of which a significant proportion presumably arises from plant-based foods.

An emerging aspect of foodborne illness is the regional difference between North America and Europe. Despite similarities in geography and primary production systems there appears to be a significant difference in the incidence of plant-based foodborne illness. Although directly comparable datasets are not available, food source attribution data show that in the USA for the period 1998–2008 46% of outbreaks and 23% of deaths were associated with produce (Painter et al. 2013), while in EU Member States for the year 2015, 17% of outbreaks were attributed to produce (EFSA 2016). This difference is of unknown cause but it could be attributed to differences in distribution systems, irrigation strategies and weather impacts. Proximity to livestock farming has been identified as an important risk factor, although it is unlikely to be a major driver for this geographical difference since livestock densities are similar, e.g. the USA reported 90 M cattle for the year 2012

(USDA 2018) while the EU Member States reported 87 M for the year 2013 (Eurostat 2018).

Management of Human Pathogens on Edible Crops

Control of human pathogens on plants relies heavily on risk management and implementation of HACCP (Hazard Analysis and Critical Control Points) principles (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards) 2014). Given the role of irrigation in transmission of pathogens, various guidelines and regulations are in place for microbial quality of the water. In addition, farm management practices are put in place to protect edible crops from wildlife (Gil et al. 2015). Plant age appears to be an important factor for the ability of human pathogens to colonise plants; regulations are in place for sprouted seeds ((EU) No 209/2013; (EU) No 208/2013) but not for young plants marketed as micro-leaf, which are at high risk of colonisation (Wright and Holden 2018). Contamination can also occur post-harvest, during the production process. Appropriate risk management systems and control procedures are therefore needed pre- and post-harvest (CFA 2007; ICMSF 2002). One aspect currently under investigation for control of plant pathogens that could also aid in food safety is in exploitation of the plant defence response. Elicitors that prime a defence response have been shown to control bacterial and fungal pathogens on plants (Wiesel et al. 2014), and appropriate targeting of the defence response could be extended to include human pathogens.

Conclusions

Investigation of the molecular basis of interactions between human pathogens and plants has developed substantially over the last 10 years. There are some striking similarities to plant-associated bacteria, including phytopathogens, but also some important differences in the outcome of the interaction. There are still important questions to be addressed, e.g. the extent of subversion of the plant defence response by human pathogens; the use of non-model, relevant edible crop foodborne species for examination of the immune response; and the focus on just two or three key species of pathogens, with little or no data for the less common pathogens. There are also important gaps relating to the wider ecology of human pathogens in the plant environment, and in relation to endemic plant microbiota. Geographical differences have also emerged; it is unclear what these are based on and how widespread they are, so there is a need for international networks. Finally, control has focused on establishing microbiological food safety criteria, which sets the field apart from plant pathology, where the aim is for crop protection. However, there are important overlaps here that could be exploited for the dual purpose of crop protection and

food safety. Therefore, both disciplines can continue to benefit from a shared dialogue and research interests.

Acknowledgements The work was funded by the Scottish Government Rural Affairs, Food And Environment Strategic Research Program. Thanks go to Kathryn Wright (James Hutton Institute) for permission to reproduce the microscopy images. Thanks also to Ashleigh Holmes (James Hutton Institute) for stimulating discussion on plant immunity. N. Holden is part of the EU-funded COST action HuPlantControl (CA16110), an international network for human pathogens on plants.

References

Allende, A., & Monaghan, J. (2015). Irrigation water quality for leafy crops: A perspective of risks and potential solutions. *International Journal of Environmental Research and Public Health, 12*(7), 7457–7477. https://doi.org/10.3390/ijerph120707457.

Alteri, C. J., & Mobley, H. L. T. (2012). Escherichia coli physiology and metabolism dictates adaptation to diverse host microenvironments. *Current Opinion in Microbiology, 15*(1), 3–9. https://doi.org/10.1016/j.mib.2011.12.004.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology, 57*, 233–266.

Beauregard, P. B., Chai, Y., Vlamakis, H., Losick, R., & Kolter, R. (2013). Bacillus subtilis biofilm induction by plant polysaccharides. *Proceedings National Academy of Sciences United States of America, 110*(17), E1621–E1630. https://doi.org/10.1073/pnas.1218984110.

Berg, G., Eberl, L., & Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology, 7*(11), 1673–1685.

Bigeard, J., Colcombet, J., & Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plant, 8*(4), 521–539. https://doi.org/10.1016/j.molp.2014.12.022.

Boller, T., & He, S. Y. (2009). Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science, 324*(5928), 742–744. https://doi.org/10.1126/science.1171647.

Brennan, F. P., Abram, F., Chinalia, F. A., Richards, K. G., & O'Flaherty, V. (2010). Characterization of environmentally persistent Escherichia coli isolates leached from an Irish soil. *Applied and Environmental Microbiology, 76*(7), 2175–2180. https://doi.org/10.1128/aem.01944-09.

Brunner, F., & Fraiture, M. (2014). Killing two birds with one stone: Trans-kingdom suppression of PAMP/MAMP-induced immunity by T3E from enteropathogenic bacteria. *Frontiers in Microbiology, 5*, 320. https://doi.org/10.3389/fmicb.2014.00320.

Buchholz, U., Bernard, H., Werber, D., Böhmer, M. M., Remschmidt, C., Wilking, H., Deleré, Y., an der Heiden, M., Adlhoch, C., Dreesman, J., Ehlers, J., Ethelberg, S., Faber, M., Frank, C., Fricke, G., Greiner, M., Höhle, M., Ivarsson, S., Jark, U., Kirchner, M., Koch, J., Krause, G., Luber, P., Rosner, B., Stark, K., & Kühne, M. (2011). German outbreak of Escherichia coli O104:H4 associated with sprouts. *The New England Journal of Medicine, 365*(19), 1763–1770. https://doi.org/10.1056/NEJMoa1106482.

CDC. (2018). *Multistate outbreak of E. coli O157: H7 infections linked to romaine lettuce (final update)*. Centers for Disease Control and Prevention. https://www.cdc.gov/ecoli/2018/o157h7-04-18/index.html. Accessed 21 Nov 2018.

Centers for Disease Control and Prevention. (2018a). *Multistate outbreak of cyclosporiasis linked to Del Monte fresh produce vegetable trays — United States, 2018: final update*. Atlanta: CDC.

Centers for Disease Control and Prevention. (2018b). *Multistate outbreak of cyclosporiasis linked to fresh Express Salad Mix sold at McDonald's restaurants — United States, 2018: final update*. Atlanta: CDC.

Centers for Disease Control and Prevention (CDC). (2006). Ongoing multistate outbreak of Escherichia coli serotype O157:H7 infections associated with consumption of fresh spinach-United States, September 2006. *Morbidity and Mortality Weekly Report, 55*(38), 1045–1046.

CFA. (2007). *Microbiological guidance for produce suppliers to chilled food manufacturers* (2nd ed.). Kettering: Chilled Foods Association Ltd. ISBN: 1-901798-15-1.

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nurnberger, T., Jones, J. D., Felix, G., & Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature, 448*(7152), 497–500. https://doi.org/10.1038/nature05999.

Crozier, L., Hedley, P., Morris, J., Wagstaff, C., Andrews, S. C., Toth, I., Jackson, R. W., & Holden, N. (2016). Whole-transcriptome analysis of verocytotoxigenic Escherichia coli O157:H7 (Sakai) suggests plant-species-specific metabolic responses on exposure to spinach and lettuce extracts. *Frontiers in Microbiology, 7*. https://doi.org/10.3389/fmicb.2016.01088.

Deering, A. J., Mauer, L. J., & Pruitt, R. E. (2012). Internalization of E. coli O157:H7 and Salmonella spp. in plants: A review. *Food Research International, 45*(2), 567–575. https://doi.org/10.1016/j.foodres.2011.06.058.

Desper, R., & Gascuel, O. (2004). Theoretical foundation of the balanced minimum evolution method of phylogenetic inference and its relationship to weighted least-squares tree fitting. *Molecular Biology and Evolution, 21*(3), 587–598. https://doi.org/10.1093/molbev/msh049.

Dickman, M. B., & de Figueiredo, P. (2011). Comparative pathobiology of fungal pathogens of plants and animals. *PLoS Pathogens, 7*(12), e1002324. https://doi.org/10.1371/journal.ppat.1002324.

Dixit, P. D., Pang, T. Y., & Maslov, S. (2017). Recombination-driven genome evolution and stability of bacterial species. *Genet, 207*(1), 281–295. https://doi.org/10.1534/genetics.117.300061.

Dong, Y., Iniguez, A. L., Ahmer, B. M., & Triplett, E. W. (2003). Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of Medicago sativa and Medicago truncatula. *Applied and Environmental Microbiology, 69*(3), 1783–1790.

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). (2014). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 2 (Salmonella Yersinia, Shigella and Norovirus in bulb and stem vegetables, and carrots). *EFSA Journal, 12*(12), 3937. https://doi.org/10.2903/j.efsa.2014.3937.

EFSA E. (2016). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal, 14*(12), 231. https://doi.org/10.2903/j.efsa.2016.4634.

Eurostat. (2018). *Agri-environmental indicator - livestock patterns European Commission*. https://ec.europa.eu/eurostat/statistics-explained/index.php/Agri-environmental_indicator_-_livestock_patterns

FAO/WHO. (2008). *Microbiology hazards in fresh leafy vegetables and herbs. Meeting report* (Microbiological rish assessment series no 14). Rome: Food and Agriculture Organisation of the United Nations, World Health Organisation.

Felix, G., & Boller, T. (2003). Molecular sensing of bacteria in plants - the highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. *The Journal of Biological Chemistry, 278*(8), 6201–6208. https://doi.org/10.1074/jbc.M209880200.

Freitag, N. E., Port, G. C., & Miner, M. D. (2009). Listeria monocytogenes – from saprophyte to intracellular pathogen. *Nature Reviews. Microbiology, 7*(9), 623–628. https://doi.org/10.1038/nrmicro2171.

Fritts, R. K., LaSarre, B., Stoner, A. M., Posto, A. L., & McKinlay, J. B. (2017). A Rhizobiales-specific unipolar polysaccharide adhesin contributes to Rhodopseudomonas palustris biofilm formation across diverse photoheterotrophic conditions. *Applied and Environmental Microbiology, 83*(4). https://doi.org/10.1128/aem.03035-16.

FSA. (2009). *Managing farm manures for food safety. Guidelines for growers to reduce the risks of microbiological contamination of ready-to-eat crops*. London: Managing Farm Manures for Food Safety. Food Standard Agency in UK.

Garcia, A. V., Charrier, A., Schikora, A., Bigeard, J., Pateyron, S., de Tauzia-Moreau, M.-L., Evrard, A., Mithöfer, A., Martin-Magniette, M. L., Virlogeux-Payant, I., & Hirt, H. (2014). Salmonella enterica flagellin is recognized via FLS2 and activates PAMP-triggered immunity in Arabidopsis thaliana. *Molecular Plant, 7*(4), 657–674. https://doi.org/10.1093/mp/sst145.

Gil, M. I., Selma, M. V., Suslow, T., Jacxsens, L., Uyttendaele, M., & Allende, A. (2015). Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. *Critical Reviews in Food Science and Nutrition, 55*(4), 453–468. https://doi.org/10.1080/10408398.2012.657808.

Gomez-Gomez, L., & Boller, T. (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Molecular Cell, 5*(6), 1003–1011. https://doi.org/10.1016/S1097-2765(00)80265-8.

Goudeau, D. M., Parker, C. T., Zhou, Y., Sela, S., Kroupitski, Y., & Brandl, M. T. (2013). The Salmonella transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. *Applied and Environmental Microbiology, 79*(1), 250–262. https://doi.org/10.1128/aem.02290-12.

Grant, S. R., Fisher, E. J., Chang, J. H., Mole, B. M., & Dangl, J. L. (2006). Subterfuge and manipulation: Type III effector proteins of phytopathogenic bacteria. *Annual Review of Microbiology, 60*, 425–449.

Hao, W., Allen, V. G., Jamieson, F. B., Low, D. E., & Alexander, D. C. (2012). Phylogenetic incongruence in E. coli O104: Understanding the evolutionary relationships of emerging pathogens in the face of homologous recombination. *PLoS One, 7*(4), e33971. https://doi.org/10.1371/journal.pone.0033971.

Hirneisen, K. A., Sharma, M., & Kniel, K. E. (2012). Human enteric pathogen internalization by root uptake into food crops. *Foodborne Pathogens and Disease, 9*(5), 396–405. https://doi.org/10.1089/fpd.2011.1044.

Holden, N. (2018). You are what you can find to eat: Bacterial metabolism in the rhizosphere. In A. Schikora (Ed.), *Plant-microbe interactions in the Rhizosphere* (pp. 1–16). Caister: Academic. https://doi.org/10.21775/9781912530007.01.

Holden, N., Pritchard, L., & Toth, I. (2009). Colonization outwith the colon: Plants as an alternative environmental reservoir for human pathogenic enterobacteria. *FEMS Microbiology Reviews, 33*(4), 689–703. https://doi.org/10.1111/j.1574-6976.2008.00153.x.

Holden NJ, Pritchard L, Wright KM, Toth I (2012, May) Mechanisms of plant colonization by human pathogenic bacteria: an emphasis on the roots and rhizosphere. In: de Bruijn F (ed) Molecular microbial ecology of the rhizosphere, vol 2. Wiley-BlackwellMay 2013

Holden, N. J., Wright, F., MacKenzie, K., Marshall, J., Mitchell, S., Mahajan, A., Wheatley, R., & Daniell, T. J. (2013). Prevalence and diversity of Escherichia coli isolated from a barley trial supplemented with bulky organic soil amendments: Green compost and bovine slurry. *Letters in Applied Microbiology, 58*(3), 205–212. https://doi.org/10.1111/lam.12180.

Hugouvieux-Cotte-Pattat, N. (2016). Metabolism and virulence strategies in Dickeya-host interactions. In M. SanFrancisco & B. SanFrancisco (Eds.), *Host-microbe interactions, vol 142. Progress in molecular biology and translational science* (pp. 93–129). https://doi.org/10.1016/bs.pmbts.2016.05.006.

ICMSF. (2002). *Microorganisms in foods 7: Microbiological testing in food safety management, vol 7. Microorganisms in foods*. New York: Kluwer Academic/Plenum Publishers.

Jay, M. T., Cooley, M. B., Carychao, D., Wiscomb, G. W., Sweitzer, R. A., Crawford-Miksza, L., Farrar, J. A., Lau, D. K., O'Connell, J., Millington, A., Asmundson, R. V., Atwill, E. R., & Mandrell, R. E. (2007). Escherichia coli O157:H7 in feral swine near spinach fields and cattle, central California coast. *Emerging Infectious Diseases, 13*(12), 1908–1911.

Jibrin, M. O., Potnis, N., Timilsina, S., Minsavage, G. V., Vallad, G. E., Roberts, P. D., Jones, J. B., & Goss, E. M. (2018). Genomic inference of recombination-mediated evolution in Xanthomonas euvesicatoria and X. perforans. *Applied and Environmental Microbiology, 84*(13). https://doi.org/10.1128/aem.00136-18.

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*(7117), 323–329.

Kisluk, G., & Yaron, S. (2012). Presence and persistence of Salmonella enterica serotype Typhimurium in the phyllosphere and rhizosphere of spray-irrigated parsley. *Applied and Environmental Microbiology, 78*(11), 4030–4036. https://doi.org/10.1128/aem.00087-12.

Knodler, M., Berger, M., & Dobrindt, U. (2016). Long-term survival of the Shiga toxin-producing Escherichia coli O104:H4 outbreak strain on fenugreek seeds. *Food Microbiology, 59*, 190–195. https://doi.org/10.1016/j.fm.2016.06.005.

Koczan, J. M., Lenneman, B. R., McGrath, M. J., & Sundin, G. W. (2011). Cell surface attachment structures contribute to biofilm formation and xylem colonization by Erwinia amylovora. *Applied and Environmental Microbiology, 77*(19), 7031–7039. https://doi.org/10.1128/AEM.05138-11.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., & Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. *Plant Cell, 16*(12), 3496–3507. https://doi.org/10.1105/tpc.104.026765.

Kyle, J. L., Parker, C. T., Goudeau, D., & Brandl, M. T. (2010). Transcriptome analysis of Escherichia coli O157:H7 exposed to lysates of lettuce leaves. *Applied and Environmental Microbiology, 76*(5), 1375–1387. https://doi.org/10.1128/AEM.02461-09.

Lodwig, E., & Poole, P. (2003). Metabolism of rhizobium bacteroids. *Critical Reviews in Plant Sciences, 22*(1), 37–78. https://doi.org/10.1080/713610850.

Mark, G. L., Dow, J. M., Kiely, P. D., Higgins, H., Haynes, J., Baysse, C., Abbas, A., Foley, T., Franks, A., Morrissey, J., & O'Gara, F. (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proceedings of the National Academy of Sciences of the United States of America, 102*(48), 17454–17459. https://doi.org/10.1073/pnas.0506407102.

Marshall, J., Rossez, Y., Mainda, G., Gally, D. L., Daniell, T., & Holden, N. (2016). Alternate thermoregulation and functional binding of Escherichia coli Type 1 fimbriae in environmental and animal isolates. *FEMS Microbiol Lett*. https://doi.org/10.1093/femsle/fnw251.

Mazurkiewicz, P., Thomas, J., Thompson, J. A., Liu, M., Arbibe, L., Sansonetti, P., & Holden, D. W. (2008). SpvC is a Salmonella effector with phosphothreonine lyase activity on host mitogen-activated protein kinases. *Molecular Microbiology, 67*(6), 1371–1383. https://doi.org/10.1111/j.1365-2958.2008.06134.x.

Millet, Y. A., Danna, C. H., Clay, N. K., Songnuan, W., Simon, M. D., Werck-Reichhart, D., & Ausubel, F. M. (2010). Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *Plant Cell, 22*(3), 973–990. https://doi.org/10.1105/tpc.109.069658.

Mithani, A., Hein, J., & Preston, G. M. (2011). Comparative analysis of metabolic networks provides insight into the evolution of plant pathogenic and nonpathogenic lifestyles in Pseudomonas. *Molecular Biology and Evolution, 28*(1), 483–499. https://doi.org/10.1093/molbev/msq213.

Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., & Umbarger, H. E. (1996). *Escherichia coli and Salmonella: Cellular and molecular biology*. Washington, DC: ASM Press. ISBN: 1555810845.

Nelson, E. B. (2018). The seed microbiome: Origins, interactions. *Plant and Soil, 422*(1), 7–34. https://doi.org/10.1007/s11104-017-3289-7.

Neumann, C., Fraiture, M., Hernàndez-Reyes, C., Akum, F. N., Virlogeux-Payant, I., Chen, Y., Pateyron, S., Colcombet, J., Kogel, K.-H., Hirt, H., Brunner, F., & Schikora, A. (2014). The Salmonella effector protein SpvC, a phosphothreonine lyase is functional in plant cells. *Frontiers in Microbiology, 5*, 548. https://doi.org/10.3389/fmicb.2014.00548.

Nunney, L., Hopkins, D. L., Morano, L. D., Russell, S. E., & Stouthamer, R. (2014). Intersubspecific recombination in Xylella fastidiosa strains native to the United States: Infection of novel hosts associated with an unsuccessful invasion. *Applied and Environmental Microbiology, 80*(3), 1159–1169. https://doi.org/10.1128/AEM.02920-13.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodi-

ties by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases, 19*(3), 407–415. https://doi.org/10.3201/eid1903.111866.

Phadtare, S., & Inouye, M. (2008). The cold shock response. *EcoSal Plus, 3*(1). https://doi.org/10.1128/ecosalplus.5.4.2.

Price-Whelan, A., Dietrich, L. E. P., & Newman, D. K. (2006). Rethinking 'secondary' metabolism: Physiological roles for phenazine antibiotics. *Nature Chemical Biology, 2*(2), 71–78. https://doi.org/10.1038/nchembio764.

Pritchard, L., Glover, R. H., Humphris, S., Elphinstone, J. G., & Toth, I. K. (2016). Genomics and taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. *Analytical Methods, 8*(1), 12–24. https://doi.org/10.1039/C5AY02550H.

Qi, J., Wang, J., Gong, Z., & Zhou, J.-M. (2017). Apoplastic ROS signaling in plant immunity. *Current Opinion in Plant Biology, 38*, 92–100. https://doi.org/10.1016/j.pbi.2017.04.022.

Quilliam, R. S., Williams, A. P., & Jones, D. L. (2012). Lettuce cultivar mediates both phyllosphere and rhizosphere activity of Escherichia coli O157:H7. *PLoS One, 7*(3), e33842. https://doi.org/10.1371/journal.pone.0033842.

Ragon, M., Wirth, T., Hollandt, F., Lavenir, R., Lecuit, M., Le Monnier, A., & Brisse, S. (2008). A new perspective on Listeria monocytogenes evolution. *PLoS Pathogens, 4*(9), e1000146–e1000146. https://doi.org/10.1371/journal.ppat.1000146.

Roe, A. J., Hoey, D. E., & Gally, D. L. (2003). Regulation, secretion and activity of type III-secreted proteins of enterohaemorrhagic Escherichia coli O157. *Biochemical Society Transactions, 31*(Pt 1), 98–103. https://doi.org/10.1042/bst0310098.

Rohmer, L., Hocquet, D., & Miller, S. I. (2011). Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. *Trends in Microbiology, 19*(7), 341–348. https://doi.org/10.1016/j.tim.2011.04.003.

Rossez, Y., Holmes, A., Lodberg-Pedersen, H., Birse, L., Marshall, J., Willats, W. G. T., Toth, I. K., & Holden, N. J. (2014). Escherichia coli common pilus (ECP) targets arabinosyl residues in plant cell walls to mediate adhesion to fresh produce plants. *The Journal of Biological Chemistry, 289*, 34349–34365. https://doi.org/10.1074/jbc.M114.587717.

Rossez, Y., Wolfson, E. B., Holmes, A., Gally, D. L., & Holden, N. J. (2015). Bacterial flagella: Twist and stick, or dodge across the kingdoms. *PLoS Pathogens, 11*(1), e1004483. https://doi.org/10.1371/journal.ppat.1004483.

Scales, B. S., Dickson, R. P., LiPuma, J. J., & Huffnagle, G. B. (2014). Microbiology, genomics, and clinical significance of the Pseudomonas fluorescens species complex, an unappreciated colonizer of humans. *Clinical Microbiology Reviews, 27*(4), 927–948. https://doi.org/10.1128/cmr.00044-14.

Segonzac, C., & Zipfel, C. (2011). Activation of plant pattern-recognition receptors by bacteria. *Current Opinion in Microbiology, 14*(1), 54–61.

Senthil-Kumar, M., & Mysore, K. S. (2013). Nonhost resistance against bacterial pathogens: Retrospectives and prospects. In N. K. Van Alfen (Ed.), *Annual review of phytopathology* (Annual reviews) (Vol. 51, p. 407). Palo Alto. https://doi.org/10.1146/annurev-phyto-082712-102319.

Takeda, K., Kaisho, T., & Akira, S. (2003). Toll-like receptors. *Annual Review of Immunology, 21*(1), 335–376. https://doi.org/10.1146/annurev.immunol.21.120601.141126.

Thilmony, R., Underwood, W., & He, S. Y. (2006). Genome-wide transcriptional analysis of the Arabidopsis thaliana interaction with the plant pathogen Pseudomonas syringae pv. tomato DC3000 and the human pathogen Escherichia coli O157:H7. *The Plant Journal, 46*(1), 34–53. https://doi.org/10.1111/j.1365-313X.2006.02725.x.

Tibayrenc, M., & Ayala, F. J. (2012). Reproductive clonality of pathogens: A perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. *Proceedings National Academy of Sciences United States of America, 109*(48), E3305–E3313. https://doi.org/10.1073/pnas.1212452109.

Toth, I. K., Pritchard, L., & Birch, P. R. (2006). Comparative genomics reveals what makes an enterobacterial plant pathogen. *Annual Review of Phytopathology, 44*, 305–336.

USDA. (2018) *Census of agriculture: 2012 census Ag atlas maps*. United States Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/Publications/AgCensus/2012/Online_Resources/Ag_Atlas_Maps/. Accessed 07 Nov 2018.

Van der Linden, I., Cottyn, B., Uyttendaele, M., Vlaemynck, G., Maes, M., & Heyndrickx, M. (2013). Long-term survival of Escherichia coli O157:H7 and Salmonella enterica on butterhead lettuce seeds, and their subsequent survival and growth on the seedlings. *International Journal of Food Microbiology, 161*(3), 214–219. https://doi.org/10.1016/j.ijfoodmicro.2012.12.015.

Voigt, C. A. (2014). Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Frontiers in Plant Science, 5*, 168–168. https://doi.org/10.3389/fpls.2014.00168.

Walk, S. T., Alm, E. W., Calhoun, L. M., Mladonicky, J. M., & Whittam, T. S. (2007). Genetic diversity and population structure of Escherichia coli isolated from freshwater beaches. *Environmental Microbiology, 9*(9), 2274–2288.

Wang, L., Albert, M., Einig, E., Fürst, U., Krust, D., & Felix, G. (2016). The pattern-recognition receptor CORE of Solan` detects bacterial cold-shock protein. *Nature Plants, 2*, 16185. https://doi.org/10.1038/nplants.2016.185.

Whitworth, J. (2018). *Cucumber Salmonella outbreak strain isolated from four sites of UK company*. https://www.foodsafetynews.com/2018/08/cucumber-salmonella-outbreak-strain-isolated-from-four-sites-of-uk-company/. Accessed 07 Nov 2018.

WHO. (2018). *Food safety*. World Health Organisation. http://www.who.int/mediacentre/factsheets/fs399/en/. Accessed 07 Nov 2018.

Wiesel, L., Newton, A. C., Elliott, I., Booty, D., Gilroy, E. M., Birch, P. R. J., & Hein, I. (2014). Molecular effects of resistance elicitors from biological origin and their potential for crop protection. *Frontiers in Plant Science, 5*(655). https://doi.org/10.3389/fpls.2014.00655.

Wright, K. M., & Holden, N. J. (2018). Quantification and colonisation dynamics of Escherichia coli O157:H7 inoculation of microgreens species and plant growth substrates. *International Journal of Food Microbiology, 273*, 1–10. https://doi.org/10.1016/j.ijfoodmicro.2018.02.025.

Wright, K. M., Chapman, S., McGeachy, K., Humphris, S., Campbell, E., Toth, I. K., & Holden, N. J. (2013). The endophytic lifestyle of Escherichia coli O157:H7: Quantification and internal localization in roots. *Phytopathology, 103*(4), 333–340. https://doi.org/10.1094/PHYTO-08-12-0209-FI.

Wright, K. M., Crozier, L., Marshall, J., Merget, B., Holmes, A., & Holden, N. J. (2017). Differences in internalization and growth of Escherichia coli O157:H7 within the apoplast of edible plants, spinach and lettuce, compared with the model species Nicotiana benthamiana. *Microbial Biotechnology, 10*(3), 555–569. https://doi.org/10.1111/1751-7915.12596.

Zeidler, D., Zähringer, U., Gerber, I., Dubery, I., Hartung, T., Bors, W., Hutzler, P., & Durner, J. (2004). Innate immunity in Arabidopsis thaliana: Lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. *Proceedings National Academy of Sciences United States of America, 101*(44), 15811–15816. https://doi.org/10.1073/pnas.0404536101.

Zipfel, C., & Felix, G. (2005). Plants and animals: A different taste for microbes? *Current Opinion in Plant Biology, 8*(4), 353–360. https://doi.org/10.1016/j.pbi.2005.05.004.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G., & Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. *Nature, 428*(6984), 764–767. https://doi.org/10.1038/nature02485.

Index

A
Aerial mycobiome, 201
Alien gene introgression, 40, 43
Anderson, G.
  CIMMYT International Wheat Program, 37
  climate change, 44
  death, 37
  global food security, 44
  Green Revolution, 36, 37
  humanist, scientist, and administrator, 36
  Indian Council of Agricultural Research, 36
  leadership, 37
  multidisciplinary policy, 44
  Rockefeller Foundation, 36
  Royal Canadian Air Force, 36
  teamwork, 37
  University of Saskatchewan, 36
  wheat breeder *cum* pathologist, 36
Anderson Lecture, 35
Aphis gossypii, 54
Artificial intelligence, 205, 218, 219, 227
Ash dieback, *see Hymenoscyphus fraxineus*

B
Bahia bark scaling of citrus, 54
Banana
  Cavendish, 22
  crop loss, 28
  cultivars, 23
  extinction?, 22
  genetic modification, 28
  genome editing, 28
  global production, 22
  Gros Michel, 23
  polyploidy, 27
  somaclonal variation, 27
  transgenic resistance, 28
Barley yellow dwarf virus, 38
Bill and Melinda Gates Foundation, 240
Biological control, 145
Bipolaris oryzae, 196, 201, *see Cochliobolus miyabeanus*
Black Sigatoka, *see Mycosphaerella fijiensis*
Breakfast
  food security, 216

C
CABI, 232
  food security, 232, 244
  plantwise, 232, 244
  smallholder farmers, 232, 244
Canadian Phytopathological Society, 44
Candidatus Liberibacter africanus (CLaf), 12
Candidatus Liberibacter americanus (CLam), 12
Candidatus Liberibacter asiaticus (CLas), 12
  detection, 217
  image analysis, 223
  immune tissue print assay, 216–218
  in phloem, 217
  machine learning, 223
  PCR, 217
  serological detection, 218
  See also Huanglongbing (HLB)

© Springer Nature Switzerland AG 2021

P. Scott et al. (eds.), *Plant Diseases and Food Security in the 21st Century*, Plant Pathology in the 21st Century 10, https://doi.org/10.1007/978-3-030-57899-2

Cassava brown streak virus, 48
Castanea sativa, 173
  iconic, 174
Charles Darwin, *see* Darwin, Charles
Chilo suppressalis, 99
CIMMYT
  See also International Maize and Wheat Improvement Center (CIMMYT)
CIMMYT International Wheat Program, 37
  disease resistance, 42
  international experimental wheat seed nurseries, 42
  national and regional programs, 39
  surveillance nurseries, 38
  training program, 38, 40
CIP, *see* International Potato Center (CIP)
Citrus canker, 48, 50
Citrus chlorotic dwarf virus, 227
Citrus greening, *see* Huanglongbing (HLB)
Citrus tristeza virus, 55, 227
CLas, *see Candidatus* Liberibacter asiaticus (CLas)
Climate change, 44, 49, 88, 98, 107, 110, 117, 118, 130, 146, 147, 197
  crop production, 128, 146
  pathogen range, 128, 146
Cnaphalococris medinalis, 99
Cochliobolus heterostrophus, 59
Cochliobolus miyabeanus, 99, 107
  crop loss, 201
  diagnosis, 202
  life cycle, 202
  molecular detection, 202
  monitoring, 202
  PCR detection, 203
  seed testing, 202
Codex Alimentarius, 254
Codex Committee on Pesticide Residues, 257
Coffee leaf rust, *see Hemileia vastatrix*
Coffee Rust Global Development Alliance, 16
Computer microsopes
  attractive to farmers, 239
  extension, 239
Computing environment, 220
Convolutional neural networks (CNNs), trained, 218, 219, 221, 227
Crop loss, 29, 38, 42, 44, 49, 77, 78, 82, 87, 98, 99, 101, 103, 104, 106–108, 113, 114, 116, 129, 132–144, 146, 148, 149, 162, 164, 167, 168, 170, 176–178, 180, 181, 183, 197, 201, 203, 216, 232
  attainable yield, 101
  diseases, pests and weeds, 98
  metrics, 101
  non-parametric statistics, 104, 113
  parametric statistics, 104
  representativeness, 101
  surveying farmers' fields, 102, 110
  yield *vs*. accuracy, 103
Cropping practices, 148
Cryphonectria parasitica, 48, 165, 171
  Castanea sativa, 173
  hypovirulence, 171
  impact, 171, 173, 174
  quarantine, 173
Cucumber mosaic virus (CMV), 77
Cyclospora cayetanensis, 260

D
Darwin, Charles, 8
  and *Phytophthora infestans*, 8
Diaphorina citri
  vector of *Candidatus* Liberibacter asiaticus, 216, 217
  vector of *huanglongbing*, 13
Diaporthe vaccinii, 165, 168
  impact, 168, 171
  pycnidiospores, 168
  quarantine, 169, 171
  Vaccinium, 168
Dicladispa armigera, 99
Disease diagnosis, 84–87, 197, 202, 205, 217, 232–235, 238, 239
  computational models, 227
Disease forecasting, 145, 146, 171, 181, 198, 203
Disease resistance, 37, 42, 49, 75, 79, 83, 84, 87, 88, 98, 107, 138, 141, 144, 145, 149, 169, 179
  multi-location testing, 38
Dispersal kernel, 50
Ditylenchus destructor, 165, 175
  impact, 176, 177
  potato, 175
  seed certification, 177
Durable resistance, 41, 42

E
Ecosystem services, 162–164, 166, 168, 170, 171, 173, 178, 183
Emerging diseases, 5, 12, 23, 38, 48, 49, 63, 76, 88, 98, 116, 119, 120, 140, 148, 216, 232, 241
Enterobacteriaceae, 261
  as mesophiles, 261
  related to *Erwinia*, 261
Environmental impact, 163, 164, 167, 182, 216
Escherichia coli, 259
  O104 H4, 268
  O157 H7, 259
  shigatoxigenic (STEC), 260
European Union, 162, 268
  food safety databases, 268, 269
Extension, 76, 232, 235, 237–239, 244
  information and communication technology, 238, 244
  tablet computers, 240

F
Famine, 36, 44
FAO, *see* Food and Agriculture Organization of the United Nations (FAO)
FAO International Code of Conduct on Pesticide Management, 256
FCR, *see Fusarium* crown rot, wheat (FCR)
FHB, *see Fusarium* head blight, wheat (FHB)
Flavescence doree phytoplasma, 165
  impact, 179–182
  management, 179, 182
  mandatory control, 180
  spread, 178
  vectors, 178
  Vitis, 178
Foc, *see Fusarium oxysporum f. sp. cubense* (Foc)
Fomites
  for human pathogens, 260
Food and Agriculture Organization of the United Nations (FAO), 252
Foodborne pathogens, 259
  as models of plant immunity, 266
  attachment to host tissue, 262
  cross-kingdom PAMP perception, 264
  establishment and metabolism, 266
  evolution towards plant adaptation, 262
  genomic comparisons with plant bacteria, 261
  geographical differences in occurrence, 268
  induce immune responses in animal and plant eukaryotes, 264
  localization in plant tissues, 261
  occurrence in Europe vs North America, 268
  PAMP-triggered immunity (PTI), 263
  pathogen/microbe-associated molecular pattern response (PAMP/MAMP), 263
  phylogenetic alignment of *csp* gene sequences, 264
Food safety, 29, 128, 131, 138, 140, 142, 147, 149, 162, 251–257, 268, 269
  regulation, 252, 255
Food security, 5, 6, 22, 35, 44, 48, 63, 74, 87, 98, 119, 128, 129, 131, 138–141, 143, 144, 147–149, 162, 197, 205, 217, 232, 243, 244, 256
  vs. food safety, 252
  goals, 128
  multidisciplinary policy, 37, 44
Free Trade Agreements (FTA), 254
Fungicides, 21, 49, 141, 144–146, 148, 168, 170, 204
Fusarium asiaticum, 140
Fusarium avenaceum, 138, 139, 142
Fusarium crown rot, wheat (FCR), 128
  crop loss, 129, 137, 140, 141
  cropping practices, 130
  disease resistance, 144
  food security, 143, 144
  fungicides, 144
  Fusarium acuminatum, 131
  Fusarium avenaceum, 131
  Fusarium crookwellense, 131
  Fusarium culmorum, 131
  Fusarium graminearum, 131
  Fusarium poae, 131
  Fusarium pseudograminearum, 131
  global distribution, 140, 141, 143
  life cycle, 132
  local distribution, 144
  microwaves to reduce inoculum, 145
  rotation, 144
  saprophytic survival, 144
  tillage, 144
  water stress, 132
Fusarium culmorum, 138, 139, 141, 142
Fusarium fujikuroi, *see Gibberella fujikuroi*

Fusarium graminearum, 138–142
chemotypes, 146
Fusarium head blight, wheat (FHB), 128
community profiling, 145
crop loss, 129, 135, 140, 141, 143
cropping practices, 130
deoxynivalenol, 131
disease resistance, 144
food security, 143
fungicides, 144
Fusarium avenaceum, 130
Fusarium culmorum, 130
Fusarium graminearum, 130
global distribution, 135, 140, 141
glucosyltransferase from barley, 145
life cycle, 130
local distribution, 144
management, 144
Microdochium nivale, 130
mycotoxins, 131
nivalenol, 131
rotation, 144
saprophytic survival, 144
tillage, 144
trichothecenes, 131
Fusarium langsethiae, 147
Fusarium oxysporum f. sp. *cubense* (Foc), 21
breeding programmes, 27
chlamydospores, 24
dissemination, 25
epidemiology, 22
fungicides, 26
history, 22
impact, 22
management, 22
origin, 22
pathogen-free propagation materials, 25
quarantine, 26
races, 24
resistant cultivars, 26
spread, 23
survival in soil, 24
survival on weeds, 24
TR4, 22–28
Fusarium poae, 139
Fusarium pseudograminearum, 138, 140, 142
Fusarium putrefaciens, 169
Fusarium wilt of banana, *see Fusarium oxysporum* f. sp. *cubense* (Foc)

G

Games
as part of training, 238
Genetic modification, 10, 13, 84, 87, 145, 146, 148, 149
Genome editing, 28
Germ theory of disease, 7
Gibberella fujikuroi, 196
crop loss, 203
fungicides, 204
LAMP assay, 205
life cycle, 203
molecular detection, 204
PCR detection, 204
seed certification, 205
seed dressing, 204
species complex, 204
symptoms, 203
Glenn Anderson, *see* Anderson, G.
Global Open Data for Agriculture and Nutrition (GODAN), 245
Great Bengal Famine, 201
Green Revolution, 35, 37, 44, 110, 120
Grosse Ile
mass burial site, 6

H

HACCP, *see* Hazard Analysis and Critical Control Points (HACCP)
Hazard Analysis and Critical Control Points
human pathogens, 269
Hemileia vastatrix, 48
arms race between host and pathogen, 17
effects on livelihoods, 16
factors favouring, 16
life cycle, 14
management, 17
name, 14
races, 17
HLB, *see* Huanglongbing (HLB)
Huanglongbing (HLB), 11, 48, 54, 216
automated detection, 216
detection by dogs, 226
diagnosis, 217
distribution, 12, 216
environmental impact, 216
global threat, 11, 216
hyperspectral imaging, 226
image analysis, 216
life cycle, 217
machine learning, 216
management, 14, 216
name, 11
resistance, 216
spread, 13
symptoms, 13, 217
unculturable, 216
vectors, 13
See also Candidatus Liberibacter asiaticus (CLas)

Human pathogens, 259
and crop plants, 259
management by HACCP principles, 269
management in crops, 269
species with plants as alternative hosts, 260
species with plants as fomites, 260
species with plants as main hosts, 260
transmission routes to plants, 268
Hymenoscyphus fraxineus, 54, 175

I
ICT, *see* Information and communication technology (ICT)
Immune tissue print assay, 216
Impact of plant diseases, 22, 36, 38, 42, 48, 79, 98, 120, 128, 130, 132–137, 143, 144, 149, 216–218
on crop production, 48, 162
on crop quality, 162, 164
on ecosystem services, 48, 162, 168, 170, 174
epidemics, 162
Information and communication technology (ICT), 232, 233, 235, 238, 244, 245
adoption, 244, 245
extension, 244
Integrated pest management, 149, 232, 235, 251
game of strategy, 238
Intelligence tool, 232
International Code of Conduct on Pesticide Management, 253
International Congress of Plant Pathology, 3, 44
International Food Policy Research Institute
collaboration with Plantwise, 243
International Maize and Wheat Improvement Center (CIMMYT), 36
International Potato Center (CIP), 75
International Rice Research Institute, 98, 102
International Seed Testing Association, 198
Irish potato famine, 5, 201
Boston monument, 6

K
Klebsiella pneumoniae, 262
Knowledge tool, 232

L
Landscape-scale epidemic modelling, 48
Laodelphax striatellus, 118, 119
Leptocorisa, 99
Leptosphaeria maculans, 53
Listeria monocytogenes, 260

M
Machine learning, 205, 216, 218, 219, 221, 227
Magnaporthe grisea, *see Pyricularia oryzae*
Magnaporthe oryzae, *see Pyricularia oryzae*
Magnaporthe salvinii, 99
Maize lethal necrosis disease, 48, 242
Mega-environments, 41
Microwaves to reduce inoculum, 145
Modelling, 146, 163, 167, 173
atmospheric dispersal, 66
Atto-fox problem, 61
beta-weighted mixture of exponentials kernel, 54
control radius, 61
deterministic models, 59
dispersal kernel, 50
exponential-power kernel, 51
fat-tailed kernel, 54
Gaussian kernel, 59
genomic data, 66
hair-trigger effect, 61
inverse power-law, 51
landscape-scale, 58
meteorological data, 67
optimal control strategy, 63
remote sensing, 66
spatially explicit compartmental models, 47
stochastic models, 59
surveillance networks, 67
thin-tailed kernel, 54
trade networks, 66
Monte-Carlo simulation, 164
MRL, *see* Pesticides, maximum residue limit (MRL)
Multi-location testing, 41, 43
Musa acuminata ssp. *malaccensis*, 28
Mycosphaerella fijiensis, 21, 23, 54
Mycotoxins, 120, 128, 130, 131, 142, 146, 147, 196

N
Nilaparvata lugens, 99, 119
Nobel Peace Prize
Borlaug, Norman, 37
Nomenclature of Territorial Units for Statistics (NUTS), 164, 167
Norman Borlaug, *see* Borlaug, Norman

Novel control methods, 145
Novel diagnostic methods, 217, 227, 242
NUTS, *see* Nomenclature of Territorial Units for Statistics (NUTS)

O
OECD Declaration, 253
Open data, 245
Orange-fleshed sweetpotato, 74
Organisation for Economic Co-operation and Development (OECD), 253
Oryza, 196
  species, 196

P
Pachydiplosis oryzae, 99
PAMP/MAMP (pathogen/microbe-associated) molecular pattern response, 263
Panama disease, 48
  name, 23
  See also Fusarium oxysporum f. sp. *cubense* (Foc)
Pattern recognition receptors, 264
PCR, *see* Polymerase chain reaction (PCR)
Pectobacterium, 261
Pesticides, 107, 111, 119, 120, 251
  good agricultural practice (GAP), 253, 255
  maximum residue limit, 251, 253, 255
    acceptance, 253
    harmonization, 253
    limit of analytical quantification, 256
    monitoring, 253
    ONE-MRL concept, 257
    process, 256
    regulation, 253
    threshold, 253
    toxicity, 253
  residues, 251
  resistance, 252
Pestpoint
  collaboration with Plantwise, 243
Pest risk assessment
  case studies, 162
  modelling, 163, 166
  quantitative, 164, 166, 184
Pest risk information service, 245
Phloem-limited virus diseases
  novel diagnostic methods, 227
Phytophthora infestans, 59
  climate change, 11
  historical records, 8
  impact, 10
  life cycle, 7
  mass burial site, 6
  name, 7
  spread, 8–10
  surveillance, 11
  threat to national security, 5
Phytophthora ramorum, 48
Phytosanitary measures, 163
  prioritizing, 166
Plant clinics, 232
  data collection, 235
  social media, 242
Plant doctors, 232, 234, 237–239, 243, 244
  information and communication technology, 238
  training, 232, 238
Plant health regulation, 162
Plantix
  collaboration with Plantwise, 243
Plant pathogens
  consequences of introduction, 162–164, 180
  eradication, 50
  establishment, 184
  introduction, 162–164, 173, 174, 177, 179, 184
  management, 49
  opportunistic, 260
  professional, 264
  spread, 49, 162, 164, 165, 173, 175, 179, 182, 184, 185, 216
  surveillance, 49
Plantwise, 232
  authoritative database, 244
  data sharing, 240
  data validation, 241
  diagnosis, 239
  extension tool, 244
  integrated pest management, 238
  intelligence tool, 244
  knowledge tool, 244
  mapping, 240
  PestSmart Diagnostics package, 238
  Plantwise Factsheets for Farmers, 233
  Plantwise Factsheets Library, 237
  Plantwise Knowledge Bank, 233, 237
  Plantwise Online Management System, 234, 236
  rapid response, 241
Plum pox virus, 54
Podosphaera plantaginis, 53
Polymerase chain reaction (PCR), 86, 87, 199, 200, 203–205, 217, 224, 226

Population
Asia, 118
China, 44
food security, 44, 252
global, 128, 196
India, 43
undernourished, 128
Potato blight, *see Phytophthora infestans*
Pseudocercospora fijiensis, *see Mycosphaerella fijiensis*
Pseudomonas fluorescens, 261
Puccinia graminis, 36, 43, 48, 59
Puccinia striiformis, 43
Pyricularia grisea, 99, 197
Pyricularia oryzae, 107, 118, 196, 197
ascospores, 198
conidia, 198
crop loss, 197
grass species, 197
host specificity, 197
LAMP assay, 201
lineages, 200
molecular detection, 199, 200
monitoring, 198
PCR detection, 200
seed testing, 198
serological detection, 199
spore trapping, 198, 200

Q
Quality-declared seed classification, 76
Quantitative traits, 144
Quarantine, 85, 162

R
Recilia dorsalis, 99
Resource-poor farmers, 5
Rhizoctonia solani, 99, 107, 118
Rice, 196, 203
aerial mycobiome, 201
and global population, 196
attainable yield, 103
Cochliobolus miyabeanus, 196, 201
crop health assessment, 101
crop health trends, 117
crop loss, 98, 99, 101, 103, 104, 106–108, 113, 114, 116
disease diagnosis, 197
epidemiological modelling, 107, 114
Gibberella fujikuroi, 196, 203
impact of pests, diseases and weeds, 99, 120
injury, damage, loss, 103
pathogens, 196
production, 196
production situation, 103, 111
Pyricularia oryzae, 196
seedborne pathogens, 197
yield loss modelling, 108, 116
Rice bacterial blight, *see Xanthomonas oryzae*
Rice bakanae disease, *see Gibberella fujikuroi*
Rice black-streaked dwarf virus disease (RBSDVD), 119
Rice blast, *see Pyricularia oryzae*
Rice brown spot, *see Cochliobolus miyabeanus*
Rice false smut, *see Ustilaginoidea virens*
Rice ragged stunt and grassy stunt virus disease, 119
Rice red stripe, 119
Rice sheath blight, *see Rhizoctonia solani*
Rice stripe virus disease (RSVD), 118
Rice tungro, 99, 107
Rice tungro bacilliform virus, 99
Rice tungro spherical virus, 99
RNA silencing, 83
Rotation, 130, 140, 141, 144

S
Salmonella enterica, 260
non-typhoidal, 260
Scaphoideus titanus
vector of Flavescence doree phytoplasma, 179
Scirpophaga incertulas, 99
Scirpophaga innotata, 99
Sclerotinia sclerotiorum, 67
Seed certification, 76, 175, 177, 198, 205
Sesamia inferens, 99
Slow-rusting, 42
Smallholder farmers, 16, 74, 76, 102, 106, 110, 197, 202, 232, 233, 239
Sogatella furcifera, 99, 119
Soil fertility, 44, 113
Southern rice black-streaked dwarf virus disease, 119
Spatially explicit compartmental models, 54
Spiroplasma citri, 227
Spore trapping, 198
SPS Agreement, 254
animal and plant health regulation, 254
food safety, 254
STEC, *see Escherichia coli* shigatoxigenic (STEC)

Sweet potato, 74
begomoviruses, 77
beta-carotene, 74
commensal or endosymbiotic viruses, 79
decentralized, trained vine multipliers (DVMS), 76
early-generation seed (EGS) production, 76
global distribution of viruses, 80
heterosis, 75
LAMP assay, 85, 86
molecular markers, 75
NCM ELISA, 85
protein target for resistance breeding, 82
qPCR, 85
quality-declared seed classification, 76
reference genome, 83
resistance breeding, 83
RNA silencing, 80, 84
small RNAs (sRSA), 78, 84
tube arrays, 86
virome, Africa, 78
virus cross-protection, 79
virus detection, 84
viruses reported to infect, 77
viruses synergistic with other viruses, 77
viruses unique to *Ipomoea*, 77
virus-free planting material, 76, 85
virus-resistant varieties, 75
virus synergistic interaction, 79
virus vectors, 77
white-fleshed, 74
yellow-fleshed, 74
yield losses to viruses, 77
Sweet potato chlorotic fleck virus (SPCFV), 77
Sweet potato chlorotic stunt virus (SPCSV), 75
Sweet potato collusive virus (SPCV), 77
Sweet potato C6 virus (SPC6V), 77
Sweet potato feathery mottle virus (SPFMV), 75
Sweet potato mild mottle ipomovirus (SPMMV), 80
Sweet potato mild mottle virus (SPMMV), 77
Sweet potato pakakuy virus (SPPV), 78
Sweet potato symptomless mastrevirus, 78
Sweet potato vein clearing virus (SPVCV), 77
Sweet potato virus 2 (SPV2), 77
Sweet potato virus C (SPVC), 77
Sweet potato virus disease (SPVD), 74
Sweet potato virus G (SPVG), 77
Synthetic hexaploid wheat, 41

T
Training of trainers, 232
Trangenics, *see* Genetic modification
Trioza erytreae, 13
Tuta absoluta
Kenya, 243

U
UN Agenda for Sustainable Development, 128
United Nations Statistical Department, 252
Ustilaginoidea virens, 118

V
Vaccinium, 168
Viral coevolution with host plants, 83
Viral virulence factors
RNA silencing suppression (RSS) proteins, 80–82
Vitamin A deficiency (VAD), 74

W
Wheat, 128
climate change, 146
crop loss, 129
cropping practices, 148
production, 128
top 10 producing countries, 128
Wide crosses, 40
Wild plants
as reservoirs of virus diversity, 83
World food programme, 16
World Trade Organization (WTO), 254

X
Xanthomonas axonopodis, 54
Xanthomonas oryzae, 99, 107, 118
Xylella fastidiosa, 48, 262