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Fanghao Wan
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Aibin Zhan *Editors*

Biological Invasions and Its Management in China

Volume 2

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Volume 13

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Biological Invasions and Its Management in China

Volume 2

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Foreword

China is one of the countries severely affected by biological invasions. By the end of 2016, at least 610 alien invasive species (AIS) had been identified in China, 50 of which are on the list of the world's 100 worst invasive species (IUCN). AIS have caused huge economic losses, estimated to be 17 billion US dollars per year. In addition, AIS negatively influence multiple ecosystems and biodiversity, leading to large-scale degradation of invaded ecosystems. Thus, biological invasions are considered as one of the biggest biosecurity issues in China.

To cope with biological invasions, a great number of research projects have been awarded by central and local governments in China since the beginning of the twenty-first century. These projects cover all aspects of biological invasions, from surveys of species distribution and damage to conducting advanced research on the mechanisms of invasion success and developing control techniques/strategies. Great progress has been made in several fields, including identifying which species have the highest possibility of being introduced into China, why AIS are successful in invasions in different regions/environments, and where they would have a high risk of causing serious damage. Moreover, Chinese scientists have successfully developed techniques for rapid early detection and field monitoring, and most importantly, effective control methods have been developed for highly invasive species. So far, we have clarified the mechanisms underlying successful invasions for several AIS, such as the asymmetric mating interactions in the whitefly *Bemisia tabaci*, and communication between pinewood nematodes, insect vectors, and associated microbes.

As an outcome of extensive researches in this field, Chinese scientists have published more than 1400 papers in ISI-indexed journals. There are also a large number of papers published in Chinese journals. Furthermore, numerous field examples of successful control activities have been implemented but not reported. To present a comprehensive view of these results, the publication of an English monograph that summarizes the major findings/experiences in China is timely. The book *Biological Invasions and Its Management in China* edited by Fanghao Wan, Mingxing Jiang, and Aibin Zhan and their colleagues covers topics of current interest and research progress in the field of biological invasions in China.

The book contains several important themes, such as what invasion problems have occurred in Chinese major ecosystems, what has been done to solve current problems, and what are the major research directions in China. In addition, the book includes findings from other parts of the world to provide comprehensive information to readers. Therefore, readers will find many areas of interesting research in this book. We expect this book to appeal widely to scientists and staff who work in the field of biological invasions.

The arrival of alien species in China and their impacts will undoubtedly continue, mainly owing to increased international trade and travel. The invasions may escalate as some of the plans to open up the country are implemented in China, such as the "One Belt and One Road" (OBOR) policy. As a result, the issues of biological invasions faced by China will also affect other countries, such as those along the OBOR. It is, therefore, crucial to promote exchanges and collaborations between Chinese scientists/governments and those overseas in the field of AIS. From this point of view, this book is expected to become a very valuable shared source of information.

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Preface

During the past decades, China has been greatly challenged by a dramatic increase in alien invasive species (AIS), leading to significant negative impacts on the economy, ecology, and even social development. Many ecosystems in China have been seriously affected by AIS, including agricultural and forest ecosystems. As a consequence, biological invasions have become a major focus for scientific research and administrative management in China, in particular since the beginning of the twenty-first century.

Our purpose in editing this book stems from the research advances that have been achieved in the last two decades in the field of biological invasions in China. These advances relate not only to scientific research but also to the management of invasive species. Our book will provide readers with information on what we have already done and what we propose to do in future studies, by reviewing a large volume of research findings and management experiences in China.

Exchanges between China and other countries are increasing. China has a very diverse range of ecosystems and is developing extensive trade in multiple categories of products. These trends are predicted to persist for the next few decades, particularly after the implementation of "One Belt and One Road" strategy. Human-mediated introductions of AIS create some features of biological invasions specific to China. Clearly, studying the trend of new types of invasions, as well as the possible mechanisms underlying invasions, will contribute to the control and management of AIS and will also enhance international collaborations to mitigate the negative impacts of AIS.

Despite the enormous efforts dedicated to control, the level and rate of invasions are continuing to increase due to the dramatic growth in international trade and travel, as well as in the nationwide transport of multiple categories of products and materials. Central and local governments need to exert more efforts on regulatory and administrative activities. The public will need to increase their awareness of biological invasions and to be more extensively involved in the control of AIS. Overall, some serious invasive species have not been effectively managed and are still causing serious damage to China, while new ones are being introduced that are not yet recognized because their populations are small or they have not caused environmental problems. There is little doubt that many fundamental topics remain to be answered in invasion biology. All these problems are examined in our book, which will benefit readers who want a comprehensive understanding of the position on biological invasions and relevant research in China.

Although we aim to provide a thorough coverage of the topic to readers, this book cannot cover all aspects of biological invasions in China. Articles by Chinese scientists are being published at a rapid rate, and thus readers may find that some valuable results are not included in our book. Moreover, as biological invasion itself is a fast-growing field, readers will find that some issues in the book have not yet been solved or even examined in detail. We hope that this book will promote active discussions in the field and draw attention to the problem of invasive species in China.

This book consists of two major parts: first, Chaps. 2, 3, 4, 5 and 6 that represent biological invasions in different types of ecosystems and, second, Chaps. 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33 that address the invasion and management of representative invasive species. For each chapter, we include the results of the most relevant studies and management strategies/techniques. An index at the end of the book will help readers to find topics of interest to them. The book will be of interest to researchers, regulatory administrators, environmental managers, and the public.

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Numerous scientific findings described in our book are generated from the research projects financially supported since 2002 by the Ministry of Science and Technology (MOST), Ministry of Agriculture (MOA), National Natural Science Foundation of China (NSFC), etc., particularly the national projects of "National Key Research and Development Programs" (2016YFC1200600, 2016YFC1202100, 2016YFC1201200) and the "948 Program" (2016-X48).

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Invasion and Management of Major Alien Non-insect Animals, Plants and Microorganisms in China

Chapter 18
Pinewood Nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle

Lilin Zhao and Jianghua Sun

Abstract Pine wilt disease (PWD) is likely the most serious threat to pine forests worldwide. The causative agent of PWD, the pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, engages in a symbiotic partnership with its insect vector, the *Monochamus* beetle, as well as associated bacteria and ophiostomatoid fungi, to successfully infect and kill its host pine trees. In this chapter, we focus on the interspecific communication between PWN and its associated partners, and the potential role of this communication in promoting pathogenicity and invasiveness of PWN. We describe the chemical and molecular signals positively influencing the survival, reproduction and spread of PWN. By considering life cycle of pinewood nematode and its interactions with other biological factors, many methods have been developed and used in infested areas. Removal of killed pines is the most important method in China. Direct trapping of vector adults were also used in recent years. Finally, concluding remarks and future perspectives were discussed in the chapter.

Keywords *Bursaphelenchus xylophilus* • Pine wilt disease • Associated microbes • Invasive species • Ophiostomatoid fungi • *Monochamus*

18.1 Introduction

Pines represent one of the most important tree species in global ecosystems. They are not only a predominant component of parks, natural reserves, and urban ornamental landscapes, but also a valuable source of timber and various wood products, e.g. half of the global lumber supply is from pine trees (Williams 2005). In China,

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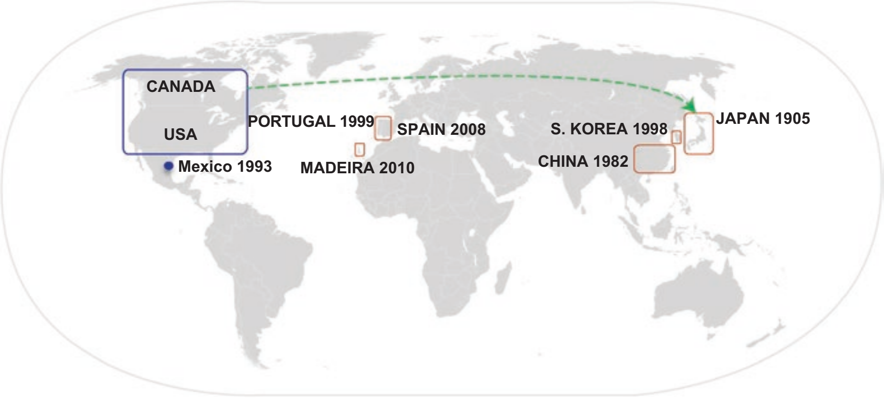

Fig. 18.1 Invasion route and distribution of PWN. The panel shows the historical spreading of PWD, from its native area (North America, *blue*) to non-native areas (Asia and Europe, *red*)

Masson pine forests, accounting for approximately 10% of the forest resource, provide the main source of wood for construction (Chen et al. 1997). The most serious threat to pine forests worldwide is pine wilt disease (PWD). PWD is caused by pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, which is native to North America and has been a deadly exotic pest of pine forests in China since 1982 (Fig. 18.1). In China, most pine species, including *Pinus massoniana*, *P. taiwanensis*, *P. yunnanensis*, *P. armandii*, *P. kesiya* var. *langbianesis* and *P. thunbergii*, can be infected by PWN (Yang et al. 2003). The infested species are different to the pine species in North America (Williams 2005).

The first PWN infection in Chinese forests was reported in Nanjing, Jiangsu Province in 1982 (Yang et al. 2003; Xu and Ye 2003). By 2007, PWN infection had been found in 113 counties of 14 provinces, with the coastal regions being most seriously affected (provinces of Zhejiang, Jiangsu, etc.) (Yu et al. 2011). The Chinese government has invested heavily in stopping the spread of PWN and minimizing timber loss (Xu and Ye 2003). However, it has killed more than one million hectares of pine forests with losses of more than US $20 billion in 15 provinces in southern China in 2011 (State Forestry Administration of the People's Republic of China 2011). It has infested to Shaanxi and Henan provinces in the northern China after 2005, suggesting that the nematode might infest more areas in the Northern Hemisphere in the future.

In addition to PWN, PWD involves several other key players, including the nematode's insect vector, the *Monochamus* beetle (Coleoptera: Cerambycidae), as well as associated bacteria and ophiostomatoid blue-stain fungi, all of which likely contribute to the pathogenicity of the nematode. PWN engages in a mutualistic partnership with the *Monochamus* beetle in order to successfully dispersal and kill its host pines. The main vector beetle is *M. alternatus* in China, different to *M. carolinensis* in North America. The new native ophiostomatoid fungi induce PWN to produce greater numbers of offspring than in North America (Hyun et al. 2007; Zhao et al. 2013b) (Table 18.1). The nematode feeds on epithelial cells of the pine tree host, as

Table 18.1 Species of PWN-associated partners by geographic region

Symbiotic species	North America	Japan	China	Korea	Europe
Vector beetle	*M. carolinensis*	*M. alternatus*	*M. alternatus*	*M. alternatus*	*M. galloprovincialis*
	M.scutellatus	*M. saltuarius*		*M. saltuarius*	
	M.mutator				
Associated bacteria	*Delftia*	*Serratia*	*Serratia* sp. M24T3	*Serratia*	*Serratia*
	Pseudomonas	*Bacillus*	*Pseudomonas*	*Brevibacterium*	*Pseudomonas*
	Stenotrophomonas	*Pseudomonas*	*Pantoea*	*Burkholderia*	*Burkholderia*
	Pantoea	*Burkholderia*	*Stenotrophomonas*	*Enterobacter*	*Enterobacter*
		Enterobacter		*Ewingella*	*Ewingella*
		Erwinia			
Symbiotic ophiostomatoid fungi	*O. ips*	*O. minus*	*O. ips*	*O. ips*	
	O. minor		*Sporothrix* sp.1		
			Sporothrix sp.2		

well as ophiostomatoid ('blue-stain') fungi that proliferate on the damaged pine tree especially during later stages of infection. The *Monochamus* beetles reside within pine trees at larval stages. During the propagative phase of life cycle, PWN reproduces rapidly, developing from the egg through four larval stages (J_1–J_4) to the reproductive adult. In this life cycle, PWN has two different long-lived, dispersal stages, the third-stage (J_{III}) and fourth-stage juveniles (J_{IV}, also referred to as 'dauer juveniles') (Pereira et al. 2013). Under unfavorable conditions such as food scarcity and low temperature, the nematode enters the dispersal phase, in which it moults from J_2 into third-stage dispersal juveniles (J_{III}). When fifth-instar larvae of the beetle enter diapause in winter, the J_{III} would aggregate around the chambers of the beetle larvae. In the following spring, once overwintered beetles develop to late pupal or young adult stages, the J_{III} would moult into fourth-stage dispersal juveniles (J_{IV}), which enter the tracheal system of the beetle ready for dispersal (Pereira et al. 2013). One adult *Monochamus* beetle can carry thousands of nematodes (1627 on average) in its tracheal system (Futai 2013). Bacterial accumulation surrounding PWN was revealed in the resin canals and along its body by transmission electronic microscopy (Suh et al. 2013). Nevertheless, the precise participation of these bacteria in the PWD disease mechanism is still a provocative issue.

With the introduction of PWN from North America to Japan in the early twentieth century and subsequently spread to other geographic locations, this pathogen has successfully exploited the lack of defenses in native pine species. Furthermore, as the nematode has existed in novel environments in Asian and European countries for ~100 years, it may have further evolved to exploit the native insect vectors, fungi, and bacteria more efficiently (Togashi and Jikumaru 2007). Therefore, we want to introduce the complex of PWN, insect vectors, fungi, and bacteria as follows.

18.2 Genetic Variation of Pinewood Nematode

In its native ranges, the pinewood nematode does not kill pine trees and rarely causes economic losses (Dwinell and Nickle 1989). The question then arises why pinewood nematode could spread uncontrollably and became highly pathogenic to pine trees when it was introduced into Asian regions. To answer this question, many studies have been conducted in China in the past 20 years. Currently, invasions and range expansion are thought to be related to genetic structure and variance (Cheng et al. 2008; Pereira et al. 2013).

18.2.1 Genetic Variation of Native and Invasive Nematode Populations

With such a successful invasive species, exploring the invasion process and its genetics is important. An amplified fragment length polymorphism (AFLP) survey was used to compare the genetic variation of native and invasive nematode populations in China and to examine the changes in genetic diversity during invasions. A total of 28 nematode populations were randomly collected from 28 geographical locations in Jiangsu, Guangdong, Chongqing in China. Chinese populations were found to have a slightly higher genetic diversity than American populations (Cheng et al. 2008). Moreover, genetic diversity differed significantly among geographic populations in China. Southern populations had a distinctly higher genetic diversity than others, and the genetic diversity of eastern coastal populations was obviously lower than that of eastern inland and western populations (Cheng et al. 2008). The 13 American populations formed a distinct group, however, the 28 Chinese samples were divided into two groups. One group was formed mainly by samples from Jiangsu, and the other mainly by samples from Zhejiang and Anhui (except samples from Mingguang, which clustered with Jiangsu). Samples from Guangdong Province were scattered across the two groups. Samples from Hubei, Guizhou and Congqing joined in the Jiangsu group. The two Japanese samples clustered with the Zhejing–Anhui group (Cheng et al. 2008). Based on the genetic relationships discovered among samples, two major invasion pathways in China are suggested. One might be from Guangdong to Anhui and Zhejiang, and the other from Guangdong to Jiangsu and then from Jiangsu to Hubei, Guizhong and Congqing. (Cheng et al. 2008). In addition, it was determined that there were no genetic bottlenecks caused by founder effect and genetic drift in Chinese populations. Multiple invasions with large amounts of nematodes from different sources led to high genetic diversity in the invasive populations. Maintenance of high genetic diversity during invasions may be one of genetic mechanisms in its invasion success. These findings provided basic background information for deep investigations to obtain a comprehensive interpretation of PWN invasion mechanisms (Xie et al. 2009).

PWN showed a low level of genetic diversity in locations where it was introduced recently, such as southwestern Europe. *Bursaphelenchus mucronatus*, an native species in the pine trees with pinewood nematodes together and an native species occurring with PWN in pine trees, displayed a high level of genetic diversity across Eurasia, possibly due to its longer time to adapt there (Pereira et al. 2013; Vieira et al. 2007). The genetic pressures of low population in PWN's pathogenic life cycle, as well as the higher dispersal capacity of its vector beetle, may have resulted in a low genetic diversity in the nematode in China and Europe (Vieira et al. 2007).

18.2.2 Defensive Mechanism in PWN Responding to Host Defenses

Genomic expansions in detoxifying and antioxidant genes in PWN have likely been driven by the nematode's need to respond to multiple layers of host defenses. PWN has numerous digestive proteases, expanded families of genes in the lysosome pathway, ATP binding cassette transporters and cytochrome P450 pathway genes (Kikuchi et al. 2011). PWN also rapidly secretes antioxidant proteins when invading a host pine. These proteins likely serve as a protective barrier that protects the nematode against a burst of reactive oxygen species generated by pine trees as a defensive mechanism during early infection. A total of 12 antioxidant proteins have been reported in the secretome of PWN, including peroxiredoxin, catalase, glutathione peroxidase, nucleoredoxin-like protein, superoxide dismutase, and thioredoxin (Shinya et al. 2013a, b). Intriguingly, the PWN secretome includes a number of proteins that potentially mimic host proteins, including two thaumatin-like proteins that show highest homology to pine tree proteins and a cystatin-like peptidase inhibitor with significant homology to a protein in the herbaceous plant *Medicago truncatula* (Shinya et al. 2013b). In plants, thaumatin-like proteins are classified as pathogenesis-related (PR) proteins and display antifungal activities by permeabilizing fungal membranes (Batalia et al. 1996). Plant cystatins play a crucial role in plant defenses against plant-parasitic nematodes (Arai et al. 2002). One of the thaumatin proteins (PR-5) and the cystatin-like peptidase (PR-6) were significantly overexpressed in susceptible pine trees during PWN infections (Hirao et al. 2012). Additionally, this phenomenon of molecular mimicry occurs in other specialized plant-parasitic nematodes, which are able to mimic endogenous host plant proteins. Wang and Lu have ever found the CLAVATA3/ESR (CLE)-like proteins using cyst nematodes (Wang et al. 2011a; Lu et al. 2009). The exact roles of the thaumatin and peptidase proteins are unknown so far; however, they probably co-evolve along with PWN and its native host pines. Because PWN invaded the pine species in Asia and Europe more recently, these pines may be more sensitive to the host mimic proteins mentioned above.

18.3 Interspecific Chemical Communication Between Pinewood Nematode, Its Insect Vector, and Associated Microbes

PWN engages in a symbiotic partnership with its insect vector, the *Monochamus* beetle, as well as associated bacteria and ophiostomatoid fungi, in order to successfully infect and kill its host pine trees. Here we focused on the interspecific communication between PWN and its associated partners, and the potential role of this communication in promoting pathogenicity and invasiveness of PWN. We describe the chemical and molecular signals positively influencing the survival,

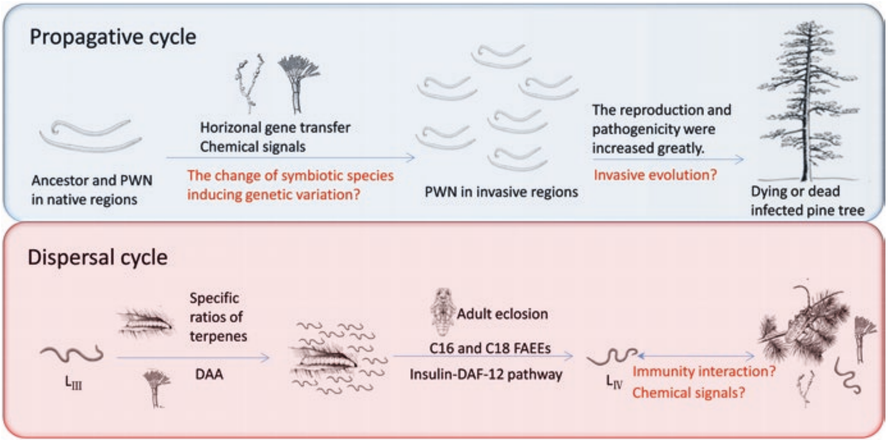

Fig. 18.2 Multispecies interactions in PWD. Upon entering a healthy pine tree, J_{IV} recover and become propagative J_n larvae, which are attracted to terpenes produced by the pine tree. PWN interacts with beneficial microbiota that may be introduced to the pine tree by the nematode, its vector beetles, or bark beetles. Blue stain fungi produce terpenes that may stimulate PWN propagation. Specific fungal isolates (*Sporothrix* sp. 1) induce the xylem tissue of the pine tree to produce diacetone alcohol (DAA), which may also increase PWN propagation and beetle larvae growth. Blue stain fungi also provide food to PWN at later stages of infestation after pine tree death. PWN may have acquired genes by HGT from associated microbiota or from pine trees that increase its pathogenicity. As conditions deteriorate inside the pine tree, PWN enters the dispersal phase of its life cycle. J_{III} form in response to unknown signals and aggregate around the pupal chamber of the vector beetle in response to terpene signals produced by the beetle larvae. Then, J_{III} molt to J_{IV} in response to C16 and C18 FAEEs released from the surface of the emerging Monochamus beetle adult. Additional chemical or other types of signals may stimulate association of J_{IV} with the beetle adult for dispersal to a new pine tree host. *Red text* indicates potential areas for future study. *Purple circles*, blue stain fungi; *green circles*, associated microbiota; *blue circles*, the pine trees; *brown circles*, insect vector; *black circles*, PWN. Abbreviations: *PWD* pinewood disease, *PWN* pinewood nematode, *HGT* horizontal gene transfer, *FAEEs* fatty acid ethyl esters

reproduction, and spread of PWN (Fig. 18.2). Knowledge of these signals could potentially be used to interfere with the proliferation and dispersal of PWN.

18.3.1 Horizontal Gene Transfer from Bacteria Benefit PWN

There is growing evidence that horizontal gene transfer (HGT) plays an important role in the evolution of nematode plant parasitism, including PWN (Davis et al. 2008; Wijayawardena et al. 2013). Genome sequencing and proteomic data mining of PWN provided strong evidence of multiple independent HGT events that potentially resulted in gene acquisition from both fungi and bacteria (Kikuchi et al. 2011; Shinya et al. 2013a). At least five groups of genes were acquired potentially from bacteria: (i) genes for a family of cell-wall degrading enzymes belonging to GH16 ß-1,3-glucanases, similar to those found in Gamma-Proteobacteria; (ii) a putative cystatin gene, which is similar to that found in Proteobacteria but divergent from PR-6 mentioned above; (iii) a gene for 6-phosphogluconolactonase similar to that from the Phylum Firmicutes; (iv) a HAD-superfamily hydrolase similar to the Firmicutes (Kikuchi et al. 2011; Shinya et al. 2013a) ; and (v) a marked expansion of genes for secreted peptidases and peptidase inhibitors (Shinya et al. 2013a). GH16 ß-1,3-glucanase is localized in the PWN's esophageal gland cells and secreted through the stylet, suggesting an important acquired feature for weakening the fungal cell walls; ß-1,3-glucan is the main structural component of the cell walls of the blue-stain fungi, thus this enzyme permits PWN to feed on fungi such as *Ophiostoma* spp. (Kikuchi et al. 2005).

The repertoire of parasitism-related genes in PWN may reflect the diversity of food sources exploited during different nematode life stages, that is, plant tissues versus fungi, and the range of critical environments that the nematode confronts, for instance, resin canals of the host trees versus the tracheae of the insect vector (Kikuchi et al. 2011). However, little is known about the functional activities and evolutionary processes of these putative genes acquired by HGT from bacteria.

18.3.2 Host Pine Volatiles Mediate PWN Reproduction and Behavior

The ratios and concentrations of terpenes differ between healthy trees and nematode-infested trees with blue-stain ophiostomatoid fungi. The terpene ratio – α-pinene, β-pinene, and longifolene at 1:0.1:0.01 – found in healthy *Pinus massoniana* xylem attracts the propagative stages of PWN (Zhao et al. 2007). In addition, the propagation rate of PWN is higher when treated with a monoterpene ratio representative of blue-stain fungi-infected pines (α-pinene : β-pinene =1 : 0.8) than when treated with a monoterpene ratio representative of healthy pines or those damaged by *M. alternatus* feeding but without blue-stain fungi (α-pinene : β-pinene =1 : 0.1) (Niu et al. 2012).

18.3.3 Chemical Signals Synchronize the Development of PWN and Its Insect Vector

J_{III} aggregation and J_{IV} formation in PWN are coordinated with the life cycle of the beetle vector (Zhao et al. 2007, 2013a; Ogura and Nakashima 2002). The J_{III} aggregate around the beetle's pupal chamber in the pine tree, possibly in response to the specific ratio of volatile terpenes – α-pinene, β-pinene, and longifolene at 1:2.7:1.1 – produced by the Monochamus beetle larvae. This terpene ratio differs from that produced by pine trees, which only attracts the propagative larval stages of the nematode (Zhao et al. 2007). Near the time of emergence of adult beetles, PWN moults into J_{IV} in response to long-chain C16 and C18 fatty acid ethyl esters (FAEEs) that are secreted from the body surface of the Monochamus beetle specifically during eclosion. Fatty acids, fatty acid methyl esters, and shorter-chain FAEEs do not induce J_{IV} formation, suggesting that the C16 and C18 FAEEs represent a specific chemical signal (Zhao et al. 2013a).

The insulin/insulin-like growth factor 1 (IGF-1) and the nuclear hormone receptor DAF-12 (dauer formation 12) pathways play a key role in dauer formation in the free-living nematode *C. elegans* and in PWN (Ogawa et al. 2009; Zhi et al. 2012; Broughton and Partridge 2009). Treatment of PWN with the PI3 kinase inhibitor LY294002 promotes J_{IV} formation, while Δ7-dafachronic acid blocks the effects of C16 and C18 FAEEs (Zhao et al. 2013a), suggesting a conserved role for the insulin/IGF-1 and DAF-12 pathways in J_{IV} formation. Transcriptional changes in the insulin/IGF-1 and DAF-12 pathways correlate with dauer entry in both *C. elegans* and PWN (Zhao et al. 2013a), indicating conservation of the transcriptional response in the two nematodes.

Several factors may explain why Monochamus beetles are the sole vector for PWN. Importantly, *Monochamus* spp. are the only beetles present at the right time and place as conditions deteriorate inside pine trees. Because J_{III} are in the center of the xylem, they are able to aggregate around the pupal chamber in response to volatile terpenes produced by the beetle larvae and subsequently develop into J_{IV} juveniles in response to the long-chain FAEEs produced by the emerging beetles. Furthermore, the beetles may secrete unidentified cues that induce the aggregation of the J_{IV} juveniles in the tracheal system of the beetle for subsequent dispersal, although this possibility is yet to be determined.

18.3.4 Host Pine-PWN and Symbiotic Fungi

Although PWN feeds mainly on plant cells during the initial infection, the nematode feeds on blue-stain fungi, such as ophiostomatoid fungi, present within the wood later in infection and after the pine host is killed. Nematode reproduction increases when blue-stain fungi are the food source. Dead wood owing to PWD is often stained blue or blue-black because these dying pine trees are also infested with bark beetles (Coleoptera: Scolytinae) that carry a wide range of ophiostomatoid (blue-stain) fungi (Zhao et al. 2008). Blue-stain fungi facilitate infection by bark beetles by decreasing the tree's vigor or by providing nutrition to bark beetles (Six et al. 2011). In addition to being carried by bark beetles, some evidence indicates that blue-stain fungi can also adhere to the body surface of adult *M. alternatus* and thus be transmitted to the twigs of healthy trees (Suh et al. 2013). Many genera of these fungi have been reported in different geographic regions (Suh et al. 2013; Wingfield 1987) (Table 18.1).

18.3.5 Associated Fungi Mediate the Development and Population of the Insect Vector

The type of fungi dominating dead wood may determine the number of PWN carried by the beetles emerging from the wood (Zhao et al. 2013b; Maehara Futai 1997). This surprising connection has been established for two fungi native to the invaded regions, namely *Ophiostoma minus* isolated in Japan and *Sporothrix* sp. 1 isolated in China (Zhao et al. 2008 ; Maehara Futai 1997). The presence of these fungi strongly and positively influenced PWN reproduction, and consequently the number of nematodes dispersed by the beetles (Zhao et al. 2008, 2013b; Maehara Futai 1997). The beetles in woods inoculated with *O. minus* carried a far greater number of PWN than those inoculated with *Trichoderma* sp. 2, *Trichoderma* sp. 3, *O. minus* and *Trichoderma* sp. (O + T), or uninoculated blocks (Maehara Futai 1997). *Sporothrix* sp. 1 positively affects the population and prevalence of the association of PWN with the native beetle *M. alternatus* in the xylem of trees (Zhao et al. 2013b). PWN produced greater numbers of offspring with a female-biased sex ratio and developed faster in the presence of *Sporothrix* sp. 1. The fragrant diacetone alcohol (DAA) released from wood infected by *Sporothrix* sp. 1 promoted fecundity of PWN and growth and survival of the beetle (Zhao et al. 2013b). That is, they determine the dynamics of PWD.

18.3.6 Host Volatiles Positively Influence Interactions of Nematode and Fungi

The terpenes released from host pine trees influence the growth of ophiostomatoid fungi identified in dead trees and the interactions of PWN and fungi (Niu et al. 2012). The monoterpenes α-pinene and β-pinene inhibited the mycelial growth of associated fungi *Sporothrix* sp.2 and *O. ips*, but had no significant effects on the growth of *Sporothrix* sp.1, which is the best food resource for PWN in China. These results suggest that the native blue-stain fungus *Sporothrix* sp.1 is not sensitive to host signals and thereby improves PWN's own propagation (Niu et al. 2012).

18.4 Monitoring and Prevention, and Semiochemical-Based Direct Control Tactics

In China, researchers pay more attention to the monitoring and preventing of the pine wilt disease, using effective and safe methods. By considering life cycle of pinewood nematode and its interactions with other biological factors, many methods have been developed and used in infested areas.

18.4.1 Spread Potential of Pine Wilt Disease in China

To determine the most appropriate control measures, it is important to anticipate where they could spread in the future. Using mathematical models to simulate the potential distribution ranges of the nematode and the disease development can help us to predict the areas at risk and consequently the areas where surveillance should be intensified.

A specific spread model was developed to describe the spread of the pine wilt disease symptoms at a larger spatial scale. It was fitted to the invasion history in China (Robinet et al. 2011), where the insect vector is mainly *M. alternatus*. It was later applied to Europe in order to determine the European ports from which the nematode and wilt disease could spread rapidly, and thus, where surveillance should be targeted more carefully (Robinet et al. 2011).

The model can simulate the potential long-distance spread of the nematode in China. The model showed that long distance dispersal appeared to be very important in China, representing more than 90% of the new infestations at an average distance of 111–339 km from the likely source populations (Robinet et al. 2009). The best factor that could explain these long-distance jumps was the human population density. This factor probably gave a good indication of the magnitude of human activity across the country and thus an indication of the amount of imports of wooden (potentially infested) materials (Robinet et al. 2009).

Climate constraints considered in the model were not the same between China and Europe. In China, the insect vector is not present throughout the country and its distribution is limited by both summer and winter temperatures (mean temperature in July above 21.3 °C and mean temperature in January above −10 °C). The only temperature constraint considered in the model applied to Europe is the temperature threshold for the disease expression (mean temperature of July above 20 °C), which is less restrictive than the temperature constrains used in China for the Asian vector. From the simulations of this model, climate change could affect more strongly the spread of the pine wilt disease in Europe than in China (Robinet et al. 2011). With 3 °C of temperature rise, the area suitable for the disease expression could expand by 40% in China against more than 100% in Europe (Robinet et al. 2011).

18.4.2 Early Detection of PWN

Early detection is of primary importance to enable rapid actions to prevent the spread and introduction of invasive species. We developed a kairomonal trapping technique that can be used to study within-tree horizontal and vertical distributions of PWN in infested stands in China (Zhao et al. 2007). It takes into account the changes of PWN within-tree distribution in relation to the development of symptoms in attacked pines. This technique provides a simple, effective, rapid and non-destructive sampling method. When 60–80% of the foliage has become pale green, PWN is recovered from larger diameter branches. As disease symptoms progress, PWN moves into and down the trunk. As the needles turn yellow, PWN was recovered from the trunk at 1–2 m above the ground (Fig. 18.3). This systematic sampling technique should greatly enhance early detection of PWN in field surveys, monitoring and phytosanitary inspections (Zhao et al. 2009).

After getting nematodes samples in trees, a real-time polymerase chain reaction (PCR) assay was developed to detect PWN. A set of primers and probes specific for PWN was designed to target the internal transcribed spacer (ITS) region. The assay was highly specific and sensitive, detecting as little as 0.01 ng of PWN DNA. The real-time PCR assay also successfully detected PWN in field samples, and it should be very useful for quarantine purposes (Cao et al. 2005). In addition, *M. alternatus* with nematodes together have also been ready for subsequent PCR detection of PWN (Wang et al. 2011b).

18.4.3 Removal of Killed Pines

The most important management tool for control is forest sanitation, especially the removal of killed pines to prevent their becoming breeding grounds for both vector beetles and the nematodes (Fig. 18.4). Entire Dead or dying trees should be removed immediately and should not be stored for later use as firewood. Even a single infected pine tree left can become an infection center that will devastate other pines nearby (Zhao et al. 2008).

In China, many treatments involving dead pines are used to halt the PWN disease cycle. The first is clear-cutting of affected areas to remove and treat dead pine trees. Secondly, heating and hydraulic pressing treatment of nematode-infected pine timbers can be conducted. Heating at 65–75 °C for 15 h, hydraulic pressing at 9 MPa, 157–168 °C for 10 min of PWN infested pine timbers (of different sizes) gave good control of both PWN and *M. alternatus*. In particular, treatment of diseased-infested boards less than 2.8-cm thick or 10 cm × 10 cm can kill 100% of the pests (Chen et al. 2000). Thirdly, the wilt-affected wood can be treated by submerging into hot water. Our trials in Nanjing showed that treating lumber with hot water to a core temperature of 60 °C for 2.30 ± 0.50 h could kill 100% of living nematodes or their beetle vectors (Zhao et al. 2008). Those methods could be used for the large-scale treatment of the trees killed by the PWN (Zhao et al. 2008).

18.4.4 Direct Trapping of Vector Adults

In China, volatiles from a stressed host have been tested for their attractions to M. alternatus. Available results showed that some components of stressed host volatiles have strong trapping ability for *M. alternatus*. Thus, using attractant-based traps are

Fig. 18.3 kairomonal trapping sampling method

Fig. 18.4 Removing killed pines from the infested forestry

Fig. 18.5 Direct trapping of vector adults using traps. The attractant named as APF-I

an effective method for monitoring beetle population dynamics and for reducing populations of *M. alternatus* in pine forests (Zhao et al. 2008).

APF-I is a popular attractant in China for attracting female beetles before oviposition and the pubescent males. It is produced in Xiamen Sanyong Co., China. This is the most effective attractant appointed by Chinese government now. The effective distance is more than 150 m, and the effective period is more than 30d (20–25 °C). In Chongqing, Sichuan, Hunan, Gaungdong and Fujian provinces, APF-1 showed the trapping efficacy more than twice higher than terpines traps (Fig. 18.5).

18.4.5 Natural Enemies

Several natural enemies have been found in *M. alternatus*. They are insect parasitoids, such as *Scleroderma guani* and *Dastarcus helophoroides*, and parasitic fungi (Liou et al. 1999), bacteria and nematodes. Parasitic fungi include *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Isaria farinosa*, *Aspergillus flavus*, *Verticillium* spp. and *Acremorium* sp., whereas parasitic bacteria include *Serratia* marcescens and the parasitic nematode, *Steinernema feltiae* (Zhao et al. 2008).

S. guani is one of the most successful natural parasites of *M. alternatus* larvae (Fig. 18.6). This parasitoid could be readily propagated on *M. alternatus* larvae. Following release of *S. guani* in pine forests, over 66.8% of *M. alternatus* larvae could be parasitized. Based on numerous field tests on release density, timing and method, the “release from a single tree method” was selected. When 5000 wasps per hectare were released using this method in a test forest in mid-July, parasitism rate

Fig. 18.6 *Scleroderma guani* were released in the forestry

Fig. 18.7 The selection of resistant pine species

of *M. alternatus* larvae was 66.82–84.21% (Fig. 18.6). This demonstrated that *S. guani* can provide good control of *M. alternatus* (Zhao et al. 2008).

18.4.6 Replanting in Clear-Cut Areas

Resistant pine species and other commercial crop were selected to protect water and soil. In clear-cut areas, there are often many openings with various size. These areas need to be reforested so that they can resume their ecological function in protecting water and soil in mountainous areas (Fig. 18.7). In a field trial beginning from 1984 in southern Nanjing, Jiangsu, two resistant pine species, including *P. taeda*, and three resistant Masson pine provenances, GX2, GX3 and GD5, were outplanted into plantations. By 1999, the areas replanted with *P. taeda* and GX2 provenances have reached 5340 and 1340 ha, respectively. In 2008, 24 years after the replanting, the two pines are still growing very well, and their selected resistance is stable and reliable. All these areas, which were previously damaged by the PWN, are again fulfilling their role in soil and water conservation (Li et al. 2013; Xu et al. 1999, 2004). Some special shrubs and commercial crops can also be used for replanting. For instance, tea trees might be good candidates, which can bring benefits for local people.

18.4.7 Selecting Bacteria to Reduce the Strong Pathogenicity of Chinese Pine Wood Nematodes

There are four dominant bacteria in U.S. pine wood nematode including *Delfita tsurhatensis*, *Pseudomonas putida*, *Stenotrophomonas maltophilia* and *Pantoea* sp. The pine seedlings treated with *S. maltophilia* withered more slowly than that treated with *Pseudomonas fluorescents*, a dominant bacterium in China. The four US bacteria also could live in Chinese nematodes. So this study provides a helpful reference for replacing virulent bacteria when released into the wild, in order to reduce the pathogenicity of Chinese pine wood nematodes (Zeng 2010). At present, Tianyilin Co. in Jiangsu Province can apply this bacterium to the infested forestry.

18.5 Concluding Remarks and Future Perspectives

In conclusion, Studies of multi-species interactions between PWN, its vector, and associated microbiota in China shed light on the potential factors that facilitate the propagation and spread of this invasive pathogen. Therefore, some methods developed based on the multi-species interactions in China were used in the infested regions effectively. By considering life cycle of pinewood nematode and its interactions with other biological factors, many methods have been developed and used in the infested areas. Removal of killed pines is the most important method in China. Direct trapping of vector adults is also used in recent years as a promising biocontrol tool. Selecting bacteria to reduce the strong pathogenicity of Chinese pine wood nematodes begin to show excited results in several regions. The number of infested regions changed to be less than before.

A comprehensive understanding of the biological interactions involved will continuously facilitate the prevention and management of PWD in the future. (Arai et al. 2002). Comparative genomics-, transcriptomics-, and proteomics-based approaches can be used to track the intrinsic low genetic diversity in the isolates of PWN compared with closely related native species of *B. mucronatus* isolated from different countries. Thus, the nucleotide sequences for genes related to the intrinsic invasiveness would be disturbed as a control target; (Batalia et al. 1996). Analysis of the adaptive evolution of gene expression related to reproduction, dispersal, and virulence of PWN with different beetle vectors and ophiostomatoid fungi over numerous generations is necessary. Tools to disrupt the association of the nematode with its vector, as well as their dispersal and spread, could offer a method to exploit the chemical signals involved in synchronization. (Broughton and Partridge 2009). Moulting to J_{III} is induced environmentally by deteriorating conditions in the pine tree or a lack of food resources. Additional unidentified chemical cues may also, for example, promote the propagative nematodes to moult to J_{III}. Further investigation regarding chemical communication in PWN could be equally concerned. With the increasing number of identified chemical signals, this technique may enable the development of methods to disrupt the development and behavior of PWN and its vector in the field.

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Chapter 19
Burrowing Nematode *Radopholus similis* (Cobb)

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Abstract *Radopholus similis* is an important pathogenic nematode of bananas and ornamentals. *R. similis* has a wide host range. It was introduced to China through importing of garden plants. At present, there is few reports about *R. similis* in Mainland China. But the risk of *R. similis* invasions in china has increased rapidly with the increasing international trade. In this chapter, we described the current invasion situation, epidemic distribution and adaptability of *R. similis* on bananas in China. It is essential to strengthen the supervision of plant quarantine and necessary to strengthen the control of the *R. similis* in greenhouse in China.

Keywords *Radopholus similis* • Distribution • Quarantine

19.1 Introduction

The burrowing nematode *Radopholus similis* (Cobb) is a migratory endoparasitic nematode that infects and damages plant roots, bulbs, and other underground tissues. It can infect more than 350 plants, which include a great number of economic crops. Infections by *R. similis* result in extensive damages to root systems, which show dark lesions, and a large number of cavities may be caused as the nematodes destructively migrate through host tissues (Fig. 19.1). The damaged plants usually have smaller leaves and delayed shooting, discolor, and even wilt or die.

So far, *R. similis* has spread to many major banana producing regions of the world, distributing widely in some countries of Europe, Asia, Africa, the Americas and Oceania. As it is an important pathogenic nematode of bananas and ornamentals, nearly 60 countries, regions or organizations have defined it as the quarantine pest, including the European Union, Portuguese Association for scientific

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Fig. 19.1 Banana root symptoms infected by the *R. similes* (From Xie 2006)

development, and the European and Mediterranean Plant Protection Organization (EPPO), Turkey, Argentina, Chile, Paraguay, Uruguay and China (EPPO 2008). Serious economic losses due to *R. similis* have been reported in some regions. For example, in India and Mexico, *R. similis* damage to banana production reached 30%–60%. In Florida, USA, the production of orange was reduced by 40%–70% in mid 1950, and *R. similis* was regarded as the main pathogenic factor. In Bangka of Indonesia, more than 90% black pepper plants died after the region was invaded by *R. similis* (Liu et al. 2011a). Serious losses due to *R. similis* damages have also been reported in flower industry and grapefruit production.

19.2 Introduction History and Current Distribution of *Radopholus similis* in China

In China, *R. similis* was first introduced in 1985 to Nanjing of Jiangsu province and Pinghe county of Fujian province, along with the import of banana seedlings from Philippines. In 1987, it spread to six counties (districts) and the total invaded areas reached 6700 ha In 1992, the epidemic completely extinguished, after the destroy of 8169 banana plants, 1088 citrus trees, 14,400 sugarcane, vegetables and other fruits, 200,000 citrus seedlings, 5000 olive seedlings. In 2001, *R. similis* was found again in some provinces in China, i.e., Guangdong, Fujian and Hainan. In Guangdong, the nematode was introduced during the import of flowers (*Anthurium*, pineapple, *Anthurium warocqueanum*, *Anthurium clarinervium*, gold emperor Philodendron, emerald gold emperor) from Korea and Malaysia to Guangzhou. However, the nematode was extinguished rapidly in each of these regions. In 2007, *R. similis* was isolated on *Anthurium* flower base in Haikou, Hainan province (Liu et al. 2007). Since then, there were seldom reports about this nematode in mainland China.

Importing of garden plants is the major pathway that *R. similis* was unintentionally introduced into China. Since 2000, Chinese entry-exit inspection and quarantine departments have frequently intercepted *R. similis* on *Anthurium*, aquatic plants, banana seedlings, *Calathea ornata*, Pineapple seedlings and other root bulbs that were imported from Singapore, South Korea, Malaysia, Denmark, Philippines, Holland and other countries.

At present, *R. similis* is mainly distributed in southern China, including Guangdong, Guangxi, Hainan, Yunnan, Fujian and other provinces. Besides, it also has the potential to invade more provinces, because some populations in China appear to have a high resistance to cold and would probably survive the winter in northern China (Peng and Xie 2005; Chen et al. 2008). In Beijing, for example, although the nematode cannot survive in winter outdoors, it would survive in the greenhouse under suitable temperature and other factor (e.g., hosts and soil pH; Zhang et al. 2008). Therefore, much attention is to be given to this nematode for its invasion risk in northern China.

19.3 Adaptability and Pathogenicity of *Radopholus similis* in China

19.3.1 Adaptability

Some *R. similis* populations in China were found to be tolerant to harsh environments (e.g., high or low temperature, dryness) than overseas populations reported previously, suggesting that this nematode has probably increased its adaptability after introduced into China (Chen et al. 2008; Huang et al. 2009). Moreover, some populations appeared to be able to survive even better than native soil nematodes (Huang et al. 2009).

19.3.2 Pathogenicity

R. similis populations from different hosts and geographical origins may differ in host range and pathogenicity (Li et al. 2016, and the references therein). Therefore, as for the populations introduced to China, their specific host range and pathogenicity have to be re-assayed so as to gather the information required for risk analysis. In China, so far the pathogenicity of various populations of *R. similis* intercepted from imported ornamentals has been tested on a number of plants, such as banana (Qin et al. 2009; Fu et al. 2011a, b), *Zingiber officinale*, *Saccharurn officin* (Han et al. 2009a), *Nicotiana tabacum*, *Mangifera indica* (Han et al. 2009b), water spinach (*Ipomoea aquatic*), malabar spinach (*Basella rubra*), and squash (*Cucurbita moschata*) (Li et al. 2016). These studies have bettered our understanding of the among-population variations of *R. similis* in parasitism and pathogenicity, and of the genetic basis of such variations (Li et al. 2016).

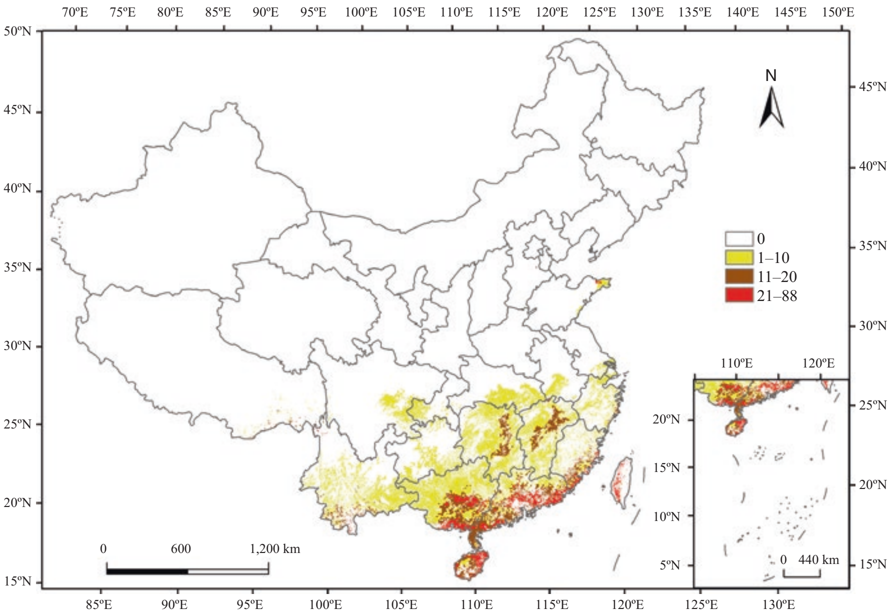

Fig. 19.2 Prediction of potential geographic distribution of *Radopholus similis* in China using MaxEnT modeling (Wang et al. 2007)

19.4 Risk Analysis

Risk analysis suggests that *R. similis* has a high invasion risk in China, with the total indicator value R reaching 2.6 (Huang et al. 2010). Potential contribution regions of *R. similis* in China were predicted by several studies. Based on the analyses using two models GARP (Genetic algorithm for rule-set prediction) and MAXENT (Maximum Entropy), Wang et al. (2007) predicted that the major regions suitable for *R. similis* were in southern China, including Hainan, Guangdong, Guangxi, Yunnan, Fujian and Taiwan provinces (Fig. 19.2). Besides, it also possibly survives in the regions in northern China, where suitable temperature and host plants are available (Zhou et al. 2005). Therefore, inspection and quarantine have to be preformed strictly to prevent the introduction and spread of this alien species. Such actions are particularly important in southern China, where orchards and horticulture would be seriously attacked in case the nematode is introduced (Peng and Xie 2005).

19.5 Techniques for Detecting and Identifying *R. similis*

R. similis has similar morphology with related species, and itself may have different morphology among the populations living under different conditions, so it is difficult and time-consuming to identify it based on morphological characteristics. For this reason, the commonly used accurate and faster identification approaches are those based on PCR, are essential for *R. similis.*

In the past decade, several primer pairs specific for rDNA-ITS region in *R. similis* have been designed (Ge et al. 2007; Liu et al. 2011b; Wang et al. 2011). Using these specific primers, *R. similis* can be reliably distinguished from other plant parasitic nematode species, including those in the genus *Pratylenchus*, *Tylenchorhynchus*, *Helicotylenchus*, *Ditylenchus*, *Filenchus*, *Criconemoides* and *Tylenchus*, and the species *Rotylenchulus reniformis* (Liu et al. 2011a, b). Moreover, some of the methods are much sensitive. For example, the methods developed by Ge et al. (2007) and Wang et al. (2011) can detect the DNA from a single individual of *R. similis*.

A LAMP method has been developed for detection of *R. similis* based on the D2-D3 of rDNA. This method was tested to be more sensitive than normal PCR-based detection methods, and the LAMP amplifications could be observed directly by eye by adding SYBR Green I and the lateral flow dipstick (LFD) (The technology can be finished in 1 hr at 63 °C. The results can be determined through color changes and observed by naked eye directly). For this reason, the LAMP assay is regarded as a highly effective, simple and economical tool for early diagnosing and monitoring of *R. slmilis*-diseased seedlings in the field. It can also be used for rapid detection of this nematode on the port line.

19.6 Management of *R. similis*

19.6.1 Quarantine

The long-distance dispersal of *R. similis* is only aided by the transfer of infested roots and the adhesion of soil of the host plant, so plant quarantine is very important. Strict examination is largely needed for the material from the epidemic area of planting vegetation, or prohibits the epidemic area of host plant planting material and soil transporting into non-endemic area. Once *R. similis* is found, effective treatment measures are in place and infested materials should be immediately destroyed. It is urgent for farmers to learn more about *R. similis* and the importance and necessity of plant quarantine, and so that they would accept quarantine consciously.

19.6.2 Prevention and Eradication

Once *R. similis* is found, it is necessary to report to the government and quarantine departments immediately, and to take timely measures to eradicate or blockade the pathogen. Strict control measures should be taken immediately to bring under control or exterminate and avoid their spread. Also, it is necessary to destroy all the infected or potentially infected plants in fields, prohibit potentially contaminated plants and soil, tools outside to prevent the epidemic from spreading. Actions should be taken to remove and destroy all plant rhizome residues in soil, then fumigate the soil with methyl bromide, Basamid or other nematicides, and cover the soil with black film, keep the soil free of weeds and any other living plants for at least 6 months. Subsequently, the establishment of an isolation belt is needed between the infection and non infection area district, where no any plants or plant roots can be exchanged between isolated zones.

19.6.3 Control Measures

The main control measures include cultural control, seed treatment and chemical control. For field crops, cultivate shallow root system plants can reduce nematode infestation. One can use warm water or nematicides to dip roots and other underground organizations tissues. In addition, it has a good control efficiency to peel off the infected section of banana seedling roots, and then soak in the nematicide liquid.

In epidemic areas, the use of nematicides is the dominant and most effective method. For the control of this nematode on banana (*Musa* AAA), Fu et al. (2012) found that nemacur (10% GR) had a high control efficiency, but it may harm plants slightly; abamectin (0.5% GR) had a control efficiency lower than nemacur, but it can promote plants' growth. Thus, they suggested using nemacur at low doses, together with abamectinm, to guarantee both control efficiency and safety. To control *R. similis* on *Anthurium andraeanum*, nemacur is also a favorable choice, and many other nematicides such as fosthiazate and temik can also be selected for use; however, macop (5% GR) should be banned, because it can exert hazard to plants (Zhang et al. 2009).

Long-term use of nematicides for *R. similis* control may bring negative impacts, such as polluting environments and increasing chemical residue in the products that are hazardous to human health (Aravind et al. 2010). So it is important to seek environmental friendly techniques. Chen et al. (2013) evaluated the predatory capacity and behavior of a mite *Blattisocius dolichus* (Acari, Blattisociidae), and suggested that this mite has the potential of becoming an effective biological agent for *R. similis*. Some bacterial isolates that are strongly antagonistic to *R. similis* have been identified from the soil, such as *Burkholderia cepacia* (Zhu et al. 2013a). *B. cepacia* was considered to be safe for human health and environments, and its antagonistic activity is stale and not affected by the ultra-violet ray (Zhu et al. 2013b).

Chinese scientists are also seeking other control methods, such as use of *R. similis*-resistant crop cultivars. Recently some banana germplasm that have high or medium resistance levels *R* have been identified (Fu et al. 2015). Scientists are also trying to find some genes in this nematode that can be targeted by novel nematicides or RNAi technique, such as cathepsin S-like cysteine proteinase gene (Lu et al. 2008), FMRFamide-like neuropeptide gene (Li et al. 2010), cathepsin B gene (Li et al. 2013; 2015a), and Calreticulin gene (Li et al. 2015a). Some of these genes have been demonstrated to be critical for *R. similis* life. For example, the cathepsin B gene plays key roles in the development, hatching and pathogenesis of *R. similis* (Li et al. 2015a), and the calreticulin gene is essential for the reproduction and pathogenicity of this nematode (Li et al. 2015b). These two genes can be the choice to be targeted for developing plant-mediated RNAi control technique for *R. similis*.

19.7 Conclusion

As the rapid development of international trade of agricultural products, *R. similis* has become a potential threat to Chinese agricultural production due to its frequent interception at ports-of-entry in china. Currently, the study of *R. similis* mostly focuses on the epidemic and adaptability of bananas and ornamentals in southern China, but few on fruits, vegetables and greenhouse vegetable in north China. In order to prevent the introduction of *R. similis* and ensure the safety of agricultural production in China, it is essential to strengthen the supervision of plant quarantine, increase input for epidemic census, notify timely the information on epidemic monitoring and dynamic, and block the spread of the epidemic completely.

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Chapter 20
Golden Apple Snails

Xiaoping Yu, Qianqian Yang, and Yipeng Xu

Abstract Golden apple snails are tremendously serious global invasive pests originated from South America. They were introduced into China in 1979 as commercial production. During last 30 years, researches on species identity, spread and origination, as well as the management have been progressed. It has been clarified that there are *Pomacea canaliculata*, *P. maculata*, and a possible cryptic species widely spread in south provinces of China. The apple snails were deduced to have a high level of diversity with multiple and secondary introductions. The invasive expansion of apple snails was affected by temperature and hydrology, but they have a high tolerance and adaption capacity. The pest control strategy, including the physical, agricultural, and biological methods are summarized, among which the biological method by releasing biological agents in crop fields has been evaluated as an effective method. Further research should focus on the spread trends and invasive pathways of different apple snail species for the integrated pest control application on field managements. The government should strengthen an administrative supervision and provide propaganda education in preventing apple snails.

Keywords Golden apple snails • Species • Spread • Management

20.1 Introduction

Golden apple snails are tropical and sub-tropical freshwater snails from the genus *Pomacea* (Mollusca: Gastropoda: Ampullariidae) (Cowie 2002). The *Pomacea* genus has dispersed explosively from South and Central America to Southeast and East Asia, and now has become a serious invader in paddy fields and other aquatic ecosystems. Two species, namely, *P. canaliculata* (Lamarck 1819) and *P. maculata* (Perry 1810) (*P. insularum* formerly), were reported as the most common invasive species. Both of them showed a tremendous invasive ability with a raising concern.

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These two species have become the major pests on aquatic crops soon after their introduction and establishment in many countries and regions (Hayes et al. 2008; Matsukura et al. 2013; Zheng et al. 2012).

In 1979, golden apple snails were introduced from Argentina to Taiwan for commercial production (Mochida 1991) and spread widely in Asia as a dietary protein supplement. However, the introduction was done in haste with no prior studies on market information and possible ecological impacts (Halwart 1994; Naylor 1996). Golden apple snails damage a wide range of plants such as algae, azolla, water hyacinth, rice seedlings, and other succulent leafy plants (Dong et al. 2011). They also destroy the aquatic ecological balances in their invaded areas and spread with a fast speed. In addition, they are infected by various parasites, especially the nematode *Angiostrongylus cantonensis*, which has been recognized as the main cause for human eosinophilic meningitis (Song et al. 2016). Golden apple snail species, as the invasive species, have aroused an increasing attention on their researches of identification, diversity, invasive pattern, and control.

20.2 Species of Apple Snails

20.2.1 Species Identification

With a very similar inter-specific and extremely intra-specific shell morphological divergence, it is difficult to distinguish *P. canaliculata* and *P. maculata* (Fig. 20.1, Hayes et al. 2012). An emerging species-level molecular framework is now beginning to bring order to the confusion of morphological taxonomy (Hayes et al. 2008, 2009, 2012; Matsukura et al. 2008).

Rawlings et al. (2007) reported that the nucleotide sequences of the mitochondrial 12S rRNA-16S rRNA and COI regions were reliable for the identification of *Pomacea* species. After combining shell and anatomy morphology and COI sequence data, Hayes et al. (2012) distinguished *P. canaliculata* and *P. maculata*, and proved *P. insularum* as the synonym of *P. maculata*. In this study, COI sequences alignment revealed that *P. maculata* and *P. canaliculata* were with an average of 2.71–2.81% divergence within populations, and of 4.80–6.87% sequence divergence between separated populations of *P. maculata* and *P. canaliculata*, respectively.

In China, Song et al. (2010) amplified COI fragments from 29 apple snails in six different locations, with the published sequences of *P. canaliculata*, *P. maculata*, *P. diffusa* (Blume 1957), *P. haustrum* (Reeve 1856), *P. paludosa* (Say 1829), *P. camena* Pain, 1949 and *Pila conica* (Wood 1828) in ampullariids. The results showed that the 619 bp COI sequences revealed 13 different haplotypes in six different locations in China. Sequence analyses, genetic distances and phylogenetic trees suggested that there were two species of non-native apple snails, namely, *P. canaliculata* and *P. maculata*, in China. It also indicated that the two species had mixed with each other in the course of pervasion. In 2013, Lv et al. obtained 521 COI sequences from

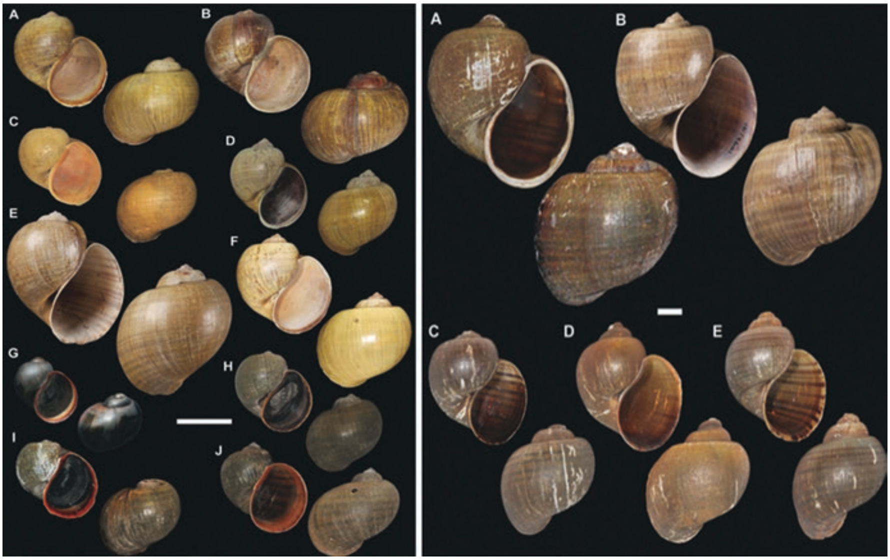

Fig. 20.1 Shell morphology of *Pomacea maculata* (*left*) and *P. canaliculata* (*right*) showing the great variation among individuals within species (Hayes et al. 2012). Scale bars: 5 mm (*left*), 1 cm (*right*)

54 sites for *Pomacea* snails. A total of 24 unique haplotypes were identified, of which 6 haplotypes were commonly found in China. Two species, *P. canaliculata* and *P. maculata*, and one phylogentically intermediate cryptic clade were detected. Bian et al. (2015) used the partial sequences of mitochondrial *atp6* genes to identify *P. canaliculata* and *P. maculata*, the intra-specific variations within *P. canaliculata* were 0–4.5%, and no sequence difference was observed within *P. maculata*, but the interspecific sequence divergence was 8.9–10.1% between the two species. Phylogenetic analyses based on sequences of *atp6* revealed that *P. canaliculata* and *P. maculata* were grouped in different clades, but the genetic trees could not reveal geographically genetic relationships of *P. canaliculata* isolates from different locations.

20.2.2 Distribution and Pest Risk Analyses

Many provinces to the south of 30 °N in China suffer the serious damage from these pests in paddy field each year (Yu et al. 2001). Pest risk analysis predicted that apple snails can occur one generation a year in more northern areas in China, probably because that they can go through winters by dormancy in silt seam of rivers (Zhou et al. 2003). In addition, filed observation revealed apple snails spread to the north of China with a speed of 8–10 km per year in the last decades (Yu et al. 2001). Now they occur in the provinces of Guangdong, Hainan, Fujian, and Guangxi regions, as

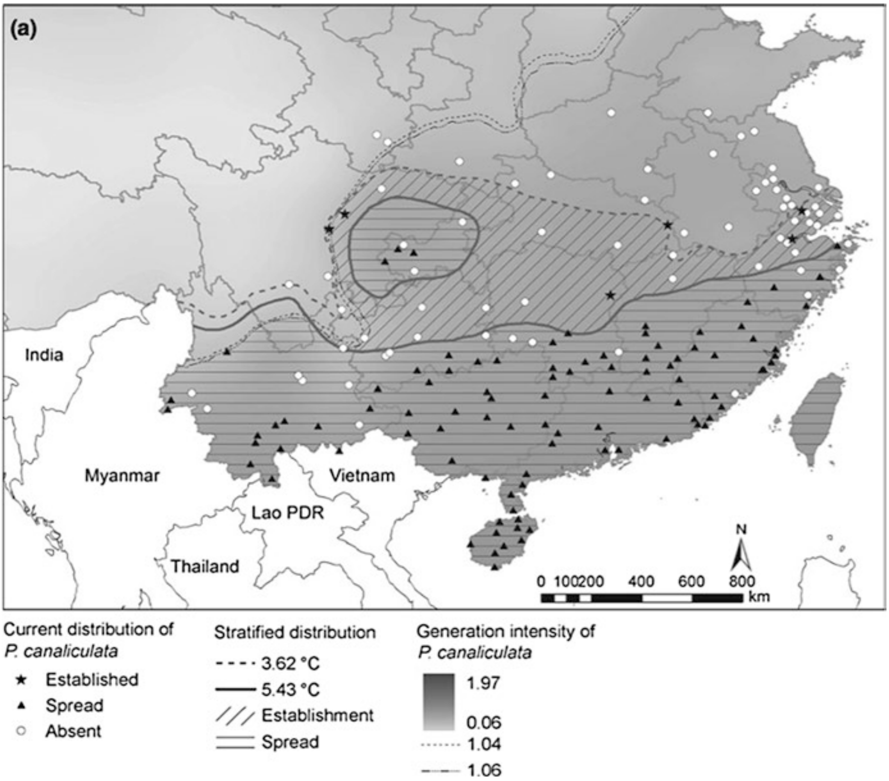

Fig. 20.2 Geographical distribution of *Pomacea canaliculata* in China. The distribution is stratified into 'spread' and 'establishment' regions (Lv et al. 2011)

well as the southern parts of Yunnan, Guizhou, Hunan, Jiangxi, and Zhejiang provinces. Isolated areas include the eastern part of Sichuan province and the western part of Chongqing municipality. A study predicted that a central region of China may be susceptible to invasion by these snails in the context of climate change (Fig. 20.2, Lv et al. 2011). Therefore, it is necessary to evaluate the risk of introduction of *Pomacea* in this region and to develop management strategies to prevent establishment and subsequent spread.

20.2.3 Origin and Genetic Diversity

Both *P. canaliculata* and *P. maculata* occur sympatrically in the Paraná and Uruguay river basins of Argentina and Uruguay, although *P. canaliculata* extends further south (Martín et al. 2001). Although *P. canaliculata* shows a wider invasive range, *P. maculata* has a much larger native range, occurring throughout much of western Brazil, from the border of Paraguay in the south to the Amazon Basin in the north, and it is a significant component of the freshwater molluscan biodiversity of the Pantanal wetlands (Hayes et al. 2012). The origins of Asian *P. canaliculata* and *P. maculata* populations have been studied to trace back to multiple locations in Argentina or to Brazil and Argentina (Hayes et al. 2008). *P. maculata* is restricted to the Sichuan basin, which signals a separate introduction from its native range or from Southeast Asia rather than from the southern part of China, where *Pomacea* snails were first reported (Song et al. 2010; Lv et al. 2013).

Generally, invasive species reveal low genetic diversity due to the foundation effects. Most studies on Chinese population genetics of *P. canaliculata* indicated low genetic diversities based on methods, such as AFLP (Xu et al. 2009)), sequence analyses of mitochondrial genes (Li et al. 2013; Hu et al. 2014), and ISSR marker (Dong et al. 2011). However, Lv et al. (2013) investigated the COI gene of apple snail species collected from 54 localities of China, and the phylogenetic analyses revealed a high genetic diversity of apple snails found in the collection sites, which also suggested multiple and secondary introductions (Fig. 20.3).

20.3 Environmental Factors Affecting the Invasion of Apple Snails

20.3.1 Low Temperature Adaption

One major factor responsible for invasion success of *Pomacea* is the environmental temperature, which can affect apple snails in various aspects, including growth, development, reproduction, feeding, and crawling behaviors (Liu et al. 2011; Seuffert and Martín 2009). Suitable temperature and fatal temperature of *P. canaliculata* are 8 ~ 38 °C, lower than 0 °C and higher than 40 °C, respectively (Pan et al. 2008; Zhou et al. 2003).

Affected by low temperature, the distribution of *Pomacea* is limited by southern or northern latitude, such as 37°S in South America or 31°N in China (Seuffert et al. 2010; Lv et al. 2011). However, *Pomacea* species can obtain acquired cold tolerance. For example, *P. canaliculata* showed a seasonal adaptation in Japan, and cold acclimation and dry condition during winter was able to enhance its cold tolerance (Matsukura and Wada 2007; Wada and Matsukura 2007; Yoshida et al. 2014). Difference in cold tolerance among *Pomacea* species has also been observed, *P. canaliculata* showing more tolerance to cold than *P. maculata* (Yoshida et al. 2014). This can explain why *P. canaliculata* has a broader distribution than *P. maculata* in the temperate region of South American and subtropical region of Asia ecosystem (Lv et al. 2013; Wada and Matsukura 2007).

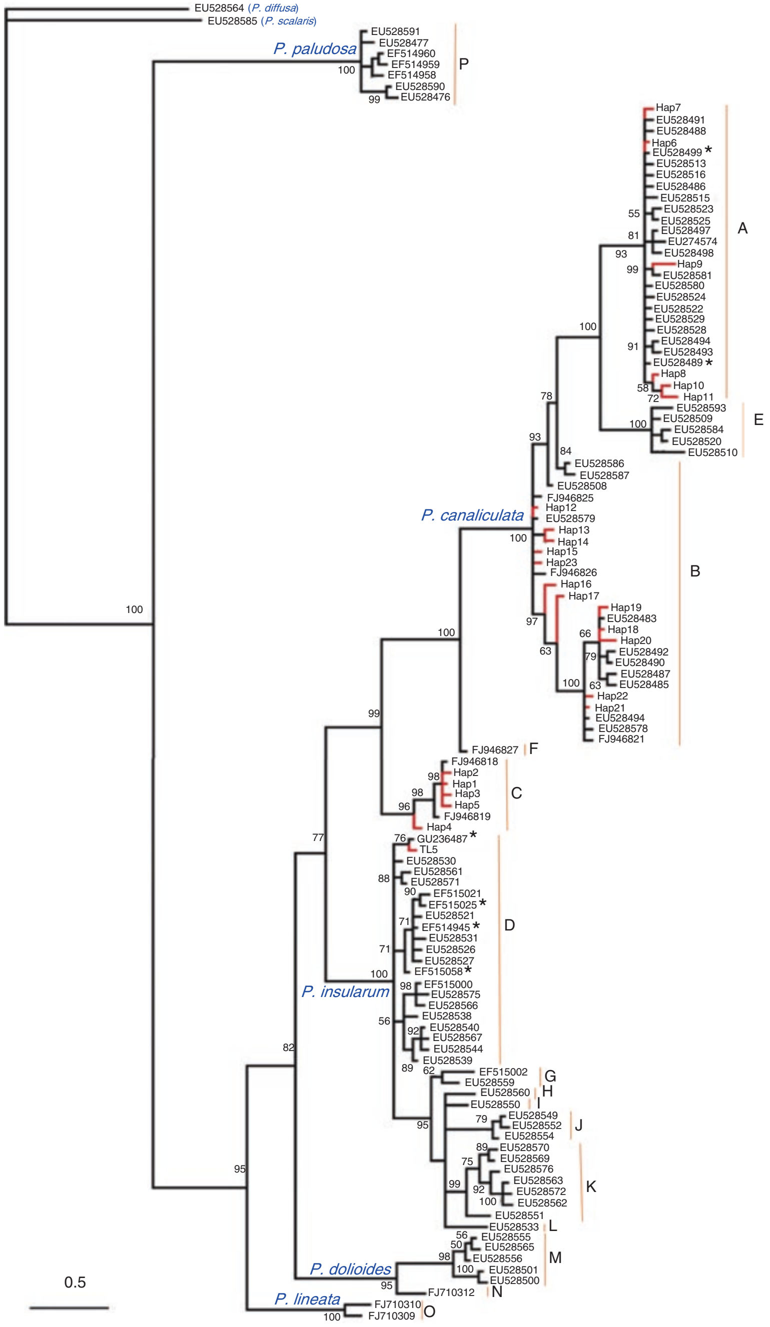

Fig. 20.3 Bayesian inference tree of *Pomacea* haplotypes. The posterior probabilities (percentage) are placed on the branches. The capital letters indicate the haplotype clusters determined by the network analysis. Asterisks denote the haplotypes that occurred in both the native and invaded ranges (Lv et al. 2013)

20.3.2 High Temperature Tolerance

Besides acquired tolerance to cold temperature, *Pomacea* also shows high tolerance to high temperature, since it has successfully spread in the Southern China and the Southeastern Asia, which belong to subtropical and tropical areas that have much higher average temperature than its native habitats in South America. *Pomacea* will estivate in response to high ambient temperature and tissue re-oxygenation during arousal from estivation will pose the problem of acute oxidative stress, but *Pomacea* can deposit uric acid as an antioxidant to deal with this problem (Giraud-Billoud et al. 2011). The quantitative proteomics and base substitution rate analyses indicated genetic basis of resistance differences to heat stress between *P. canaliculata* and its noninvasive congener *P. diffusa*, which could probably affect their further range expansion in a warming climate (Mu et al. 2015).

High temperature can induce *Pomacea* to increase the expression of heat shock proteins (HSP) (Giraud-Billoud et al. 2011; Song et al. 2014; Xu et al. 2014), which are thought to maintain other proteins in right shape under temperature stress (Kregel 2002). With heat treatment, HSP expression of *P. canaliculata* can show sex differences (Song et al. 2014). After heat shock at 36 °C, the maximal induction of *P. canaliculata* HSP70 expression appeared at 12 h and 48 h in males and females, respectively, indicating that females were more tolerant to heat than males (Song et al. 2014). Females with a higher heat resistance might be partially able to adjust to the dynamics of the sex ratio to increase the population (Song et al. 2014). The HSP expression of *P. canaliculata* induced by heat stress also showed a tissue difference (Fig. 20.4). Induced by 36 °C, both HSP70 and HSP90 expression in gill and foot were dramatically up-regulated, indicating that tissues or organs directly exposed to environment need more HSP70 and HSP90 level to protect them from thermal damage (Xu et al. 2014). However, compared with HSP90, HSP70 expression was also highly induced in the kidney of *P. canaliculata* (Xu et al. 2014).

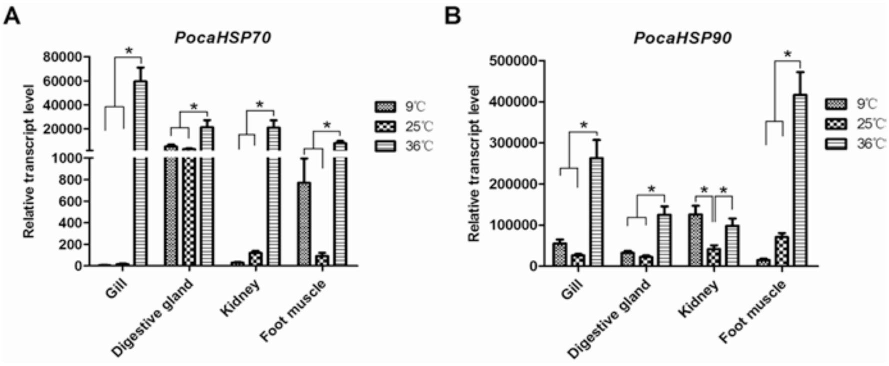

Fig. 20.4 HSP70 and HSP90 expression in the different tissues of *Pomacea canaliculata* under different temperatures. "*" represents the 0.05 significance, based on the Tukey HSD test analysis of means (n = 3). (Xu et al. 2014)

Therefore, HSP expression up-regulated variously in different tissues by heat shock might be related to the specific function of difference HSP in specific tissues.

In addition, the temperature tolerance of *Pomacea* species is influenced by the climates of its habitats. The investigation of the temperature tolerance of five *P. canaliculata* geographical groups in China showed that snails in relatively warmer area had stronger heat tolerance but weaker cold tolerance, while snails in relatively colder area showed a converse temperature tolerance (Dong et al. 2010).

20.3.3 Hydrological Factors

Besides temperature, hydrological factors, including pH, salinity, organic matter content *etc.*, may also affect the invasive process of *Pomacea* (Byers et al. 2013; Martín et al. 2001; Zhu et al. 2015; Seuffert and Martín 2013). The distribution of *P. maculata* in the Southeastern United States was limited by pH rather than climate (Byers et al. 2013). In laboratories, survival, growth, and reproduction of *P. canaliculata* also could be severely inhibited by too acidic ($pH \leq 3.5$) or too alkaline ($pH \geq 10.5$) conditions, and strongly inhibited especially if maintained under conditions lacking adequate food (Zhu et al. 2015). Salty, alkaline, poorly vegetated aquatic environments with high desiccation risk may also act as natural barriers for *Pomacea* dispersal, but integrated and flood-prone drainage systems probably attribute to the fast expansion of *Pomacea* in Southeastern Asia (Martín et al. 2001).

20.4 Management of Apple Snails

20.4.1 Traditional Control

In order to control golden apple snails, numerous measures have been taken in aquatic crop fields. Traditional apple snail managements include physical, agricultural, and chemical methods. The physical control includes, for example, handpicking egg masses and setting nets in irrigation to intercept apple snails. Apple snails have tremendous reproduction ability, producing ~1000 eggs per cluster. With a bright pink color, egg masses are easy to be found in fields. Spring and autumn are the peak seasons of reproduction, which are also the best time for controlling apple snails through picking and destroying the egg masses (Yu et al. 2001). Setting nets in irrigation can barrier apple snails spread through the flowing irrigation water networks. It showed that, a net barrier can hold up ~4000 adult apple snails (Chen et al. 2003). The agricultural control methods include, such as taking farming rotation, smash snail shells with agricultural machinery, etc. Studies revealed that, rotation of planting rice and bean can effectively reduce the population of apple snails (Wada 2004). As to the chemical methods, the molluscicide is usually used to kill apple snails with a serious environmental and human health consequence (Yu et al. 2001; Chen et al. 2003). Therefore, the chemical methods was not adopted extensively for managing apple snails.

20.4.2 Biological Control

Recently, the biological control has been applied to suppress the occurrence of apple snails by releasing biological agents such as fishes and ducks in crop fields (Liang et al. 2014). As both the apple snails and the biological agents, such as the common carp (*Cyprinus carpio*) and black carp (*Mylopharyngodon piceus*), consume the macrophytes and other animals, bio-control of apple snail populations need to consider the effects on the non-target plants and animals (Karraker and Dundgeon 2014; Ip et al. 2014).

The Chinese soft-shelled turtles, *Pelodiscus sinensis*, was tentatively selected as a new and more effective biological agent to control apple snails in *Oryza sativa* and *Zizania latifolia* field (Fig. 20.5, Dong et al. 2012). Furthermore, *P. sinensis* is of high commercial value and is commonly cultured in Malaysia, Indonesia and China for food (Jia et al. 2005).

Our results showed that the adult turtles, *P. sinensis* had a significantly higher predatory capacity for controlling snails than juvenile turtles. In the turtle-free field, the number of golden apple snail increased rapidly, especially young snails. In the turtle-released field, the number of both young and adult snails declined significantly as compared to the turtle-free field (Fig. 20.6). During first 15 days after turtles released, the number of both young and adult apple snails was hardly decreased, while the number of apple snails in tested snail-infested fields was effectively suppressed within 90 days after turtles released. Furthermore, the number of egg masses laid by apple snail was also declined significantly at the 30th day after

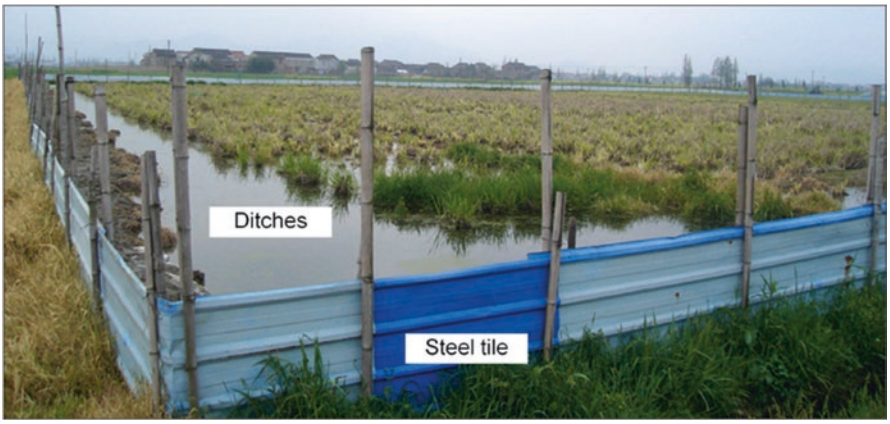

Fig. 20.5 The fences designed to enclose Chinese soft-shelled turtle *Pelodiscus sinensis* in the field with wild rice *Zizania latifolia*

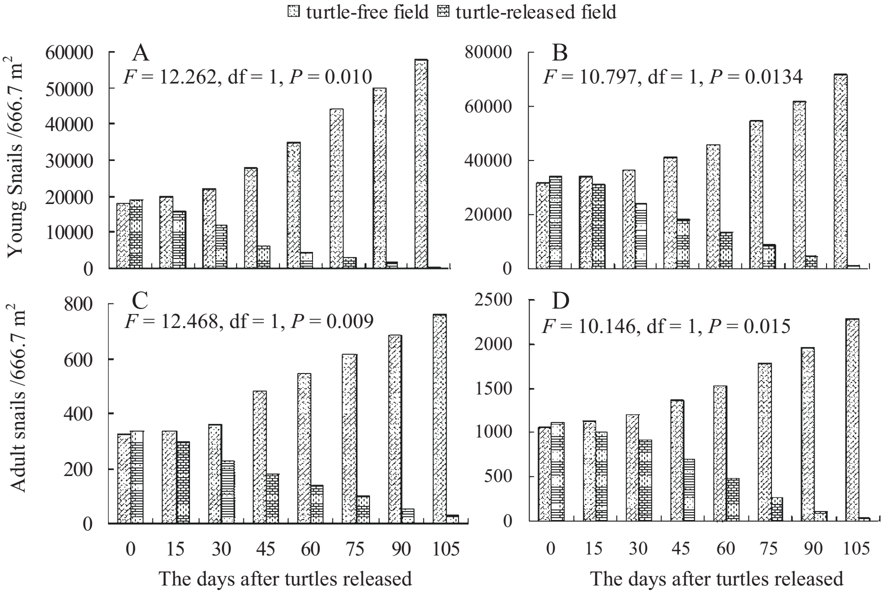

Fig. 20.6 The number of golden apple snails consumed by Chinese soft-shelled turtle *Pelodiscus sinensis* in moderately snail-infested fields (**a** and **c**) and heavy snail-infested fields (**b** and **d**). The turtles were released in June 4, 2006. The number of snails in turtle-released field and turtle-free field were compared using two-way analyses of variance (ANOVA) without replications. The tests were considered significant at $p \leq 0.05$

turtle released as compared to turtle-free field (Fig. 20.7). By the end of experiment, the new egg masses were hardly found in the turtle-released field.

In our study, we demonstrated that the Chinese soft-shelled turtle *P. sinensis* has a strong predatory capacity for controlling apple snails both in laboratory and field conditions. By the use of *P. sinensis*, an alternative method for biologically controlling apple snail was developed in *O. sativa* and *Z. latifolia* field. Keeping *P. sinensis* in rice or *Z. latifolia* field was accepted extensively by local farmers for apple snail control. In the biological control pattern, farmers can harvest from both crops and *P. sinensis*, as well as saving the cost of chemical molluscicides. By releasing turtles, the yield of *Z. latifolia* shoot increased up to 1500 ~ 2000 kg per 667 m^2 as compared to the turtle-free field without use of synthetic molluscicides, and the income per 667 m^2 increased to 800 ~ 1000 US Dollars (Fig. 20.8).

20.4.3 Strengthen Quarantine Regulation System

Since the early blind introduction as food resource and illegal carrying egg masses secretly from the native ranges of apple snail in the 1980s, human activities have played an important role in the spread of golden apple snails. It is critical to improve

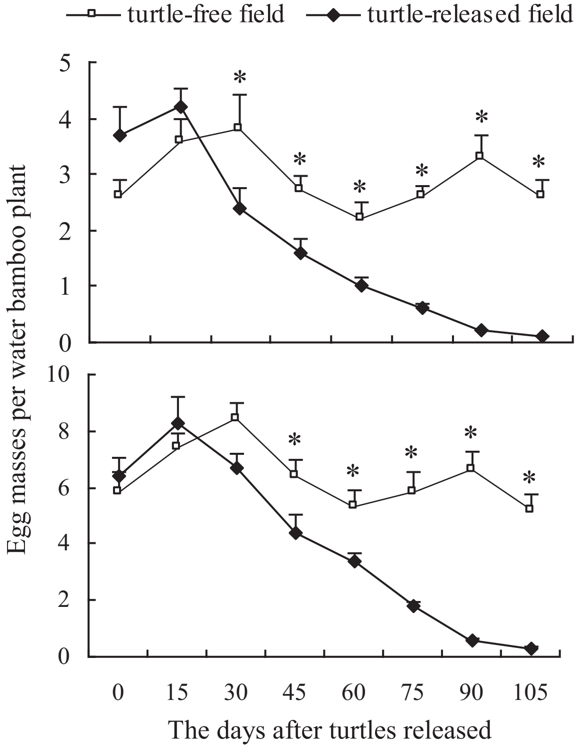

Fig. 20.7 Number of egg masses on each *Z. latifolia* plant in moderately snail-infested fields (*upper*) and heavy snail-infested fields (*below*). The Chinese soft-shelled turtles *Pelodiscus sinensis* were released in June 4, 2006. The data expressed as Means ± standard error (n = 10). At the same stage, the data with asterisks are significantly different (* $P < 0.05$) by Student's *t*-test

the invasive pest control system, especially strengthen the administrative management system. It is necessary to improve the quarantine regulations to limit the transportation of crop seedlings from the apple snail damaging regions. The apple snail damaging regions should be implemented of regional quarantine management. Furthermore, it is necessary to strengthen national public education and regulations propaganda through the media to avoid unintentional release and also to popularize quarantine laws and regulation.

For the better apple snail control, we suggest to deepen the fundamental research, including the systematic understanding on their origin, invasion mechanisms, and spread trends. In addition, we suggest promoting the applicable research, such as the pest risk analysis, species identification, pest monitoring, and pest control technique. It is necessary to combine studies on molecular identification and population genetic to monitor the national or regional damaging diversity of *Pomacea* species.

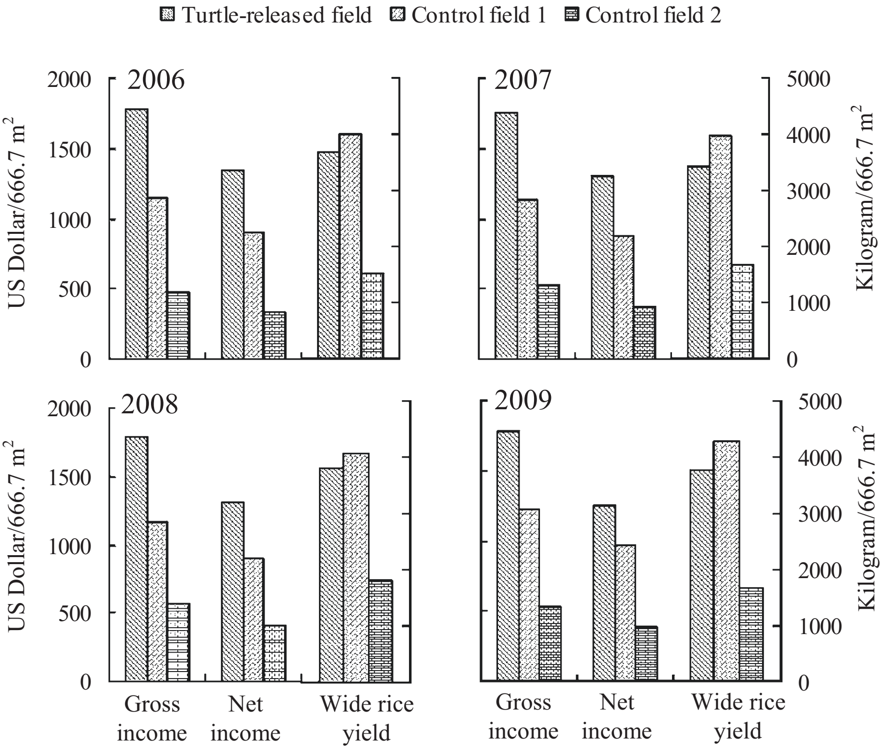

Fig. 20.8 The yield of *Z. latifolia* and farmer's income and net income between turtle-released field and turtle-free field (Control). Control field 1: the turtle-free field with use of synthetic molluscicides; Control field 2: the turtle-free field without use of synthetic molluscicides. The net income equaled to gross income minus the cost

20.5 Conclusion and Perspectives

During last over 30 years, apple snails have spread rapidly in southern China. Researchers have achieved a large progress in understanding the species, spread, and management of apple snails in China. However, studies on the invasive mechanisms and apple snail pest control are meeting with new challenges.

Firstly, the most widespread and invasive species, however, rather than the single species *P. canaliculata* as previously widely thought, has been clarified as two species, namely *P. canaliculata* and *P. maculata* (Song et al. 2010). However, a possible cryptic group observed through the phylogenetic analyses of COI sequences distributed in China (Lv et al. 2013). Since there are a number of cryptic taxa and much higher levels of diversity in their native range (Hayes 2009), then it is possible that cryptic species might be introduced. Accurate identification is important in understanding physiology, ecology, damage degree, and native range of different species, as well as in understanding invasion patterns and mechanisms, which is of significant importance in developing and implementing integrate management strategies (Hayes et al. 2008). It is critical to reveal the true identity of the cryptic apple snail group with combined morphological description and molecular characteristics. Secondly, with the global climate change, the control of apple snails in China is facing new challenges. Our sampling during August to November of 2014 has detected the distribution of apple snail in Jiangsu province, which has moved across the 30 °N as a presumed distribution limits described previously (Yu et al. 2001). The cold and hot tolerance study indicated a broad adaption of apple snail to the temperature. It is necessary to strengthen study on invasion mechanism with the more accurate monitoring of distribution of apple snail in the field. Thirdly, as a global invasive pest, study of apple snail in China should be deepened through an international collaboration, especially in fundamental research and pest management techniques. It will be necessary to build up an international apple snail control research, development and training center, establish the international collaborate network to play the advantage of different countries/regions for the better control of apple snails.

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Chapter 21
Red-Eared Slider *Trachemys scripta elegans* (Wied-Neuwied)

Kai Ma and Haitao Shi

Abstract The red-eared slider (*Trachemys scripta elegans*) is listed as one of the 100 worst invasive species in the world. At present, it is widely distributed in aquatic habitats in China. In order to collect detailed ecological data in invaded regions, to clarify the invasive status, ecological impacts and adaptative mechanisms, and to provide data for legislation and management, we conducted a series of studies on the invasion ecology of the exotic red-eared slider from 2008 to 2014 in China. The results showed that this slider species has a high ecological tolerance and behavioral plasticity, which result in a strong competitive ability and a large preponderance in local communities. The slider poses a big threat to native biodiversity and ecosystems in China. All our results suggest that we should enact laws to ban the import of sliders and to make a scientific management of turtle farms, pet markets, and animal release. In addition, the propaganda and public education should be regularly done to enhance the knowledge and public awareness of negative effects of biological invasions.

Keywords Invasive species • Red-eared slider • Population status • Adaptive mechanism • Ecological impacts

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Fig. 21.1 The red-eared slider (Photo by Kai Ma)

21.1 Introduction

The red-eared slider (*Trachemys scripta elegans*) is a medium-sized semi-aquatic freshwater turtle. It is the most common subspecies of the slider (*T. scripta*) that includes yellow-bellied slider (*T. s. scripta*), red-eared slider (*T. s. elegans*) and Cumberland slider (*T. s. troostii*) (Seidel 2002). The red-eared slider has a conspicuous wide red postorbital stripe, narrow chin stripes, a transverse yellow bar on each pleural, and a plastral pattern consisting of a dark blotch or an ocellus on each scute (Ernst 1990; Fig. 21.1).

The red-eared slider is native to eastern United States and northeastern Mexico. It occupies the Mississippi Valley from Illinois to the Gulf of Mexico, including 19 states of USA (Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Nebraska, New Mexico [eastern], Ohio, Oklahoma, Tennessee, Texas, West Virginia) and two states of Mexico (Nuevo Leon, Tamaulipas) (Ernst 1990). By the end of the Second World War, the demand for pet turtles increased dramatically, leading to the prosperous commercial turtle farming in USA and then a huge amount of the red-eared sliders exported to many countries (Bringsøe 2006). So far, this slider has been introduced into more than 70 countries and regions in Europe, Africa, Oceanica, Asia and America (Kraus 2009; van Dijk et al. 2011; Fig. 21.2). As this species is omnivorous (Collins 1982) and has an extensive ecological tolerance (Willmore and Storey 2005) and strong diffusion ability (Burke et al. 1995), it is listed as one of the 100 worst invasive species in the world (Lowe et al. 2000).

Since 1986, the red-eared slider was introduced into mainland China *via* Hong Kong, as a pet owing to its tenacious vitality, colourful body and low price (Shi et al. 2008). The number of sliders exported to China from the USA reached 4.65 million in 1998, 4.71 million in 1999, and 7.5 million in 2000 (CITES 2003). In early years of this century, a massive scale of red-eared slider farming boomed in China, for example, in 2005 to the total number of farmed red-eared sliders reached 25 million in five provinces, including Jiangsu, Zhejiang, Hunan, Guangdong and Hainan (Xu et al. 2006). So far, this slider is widely distributed in central and southern China

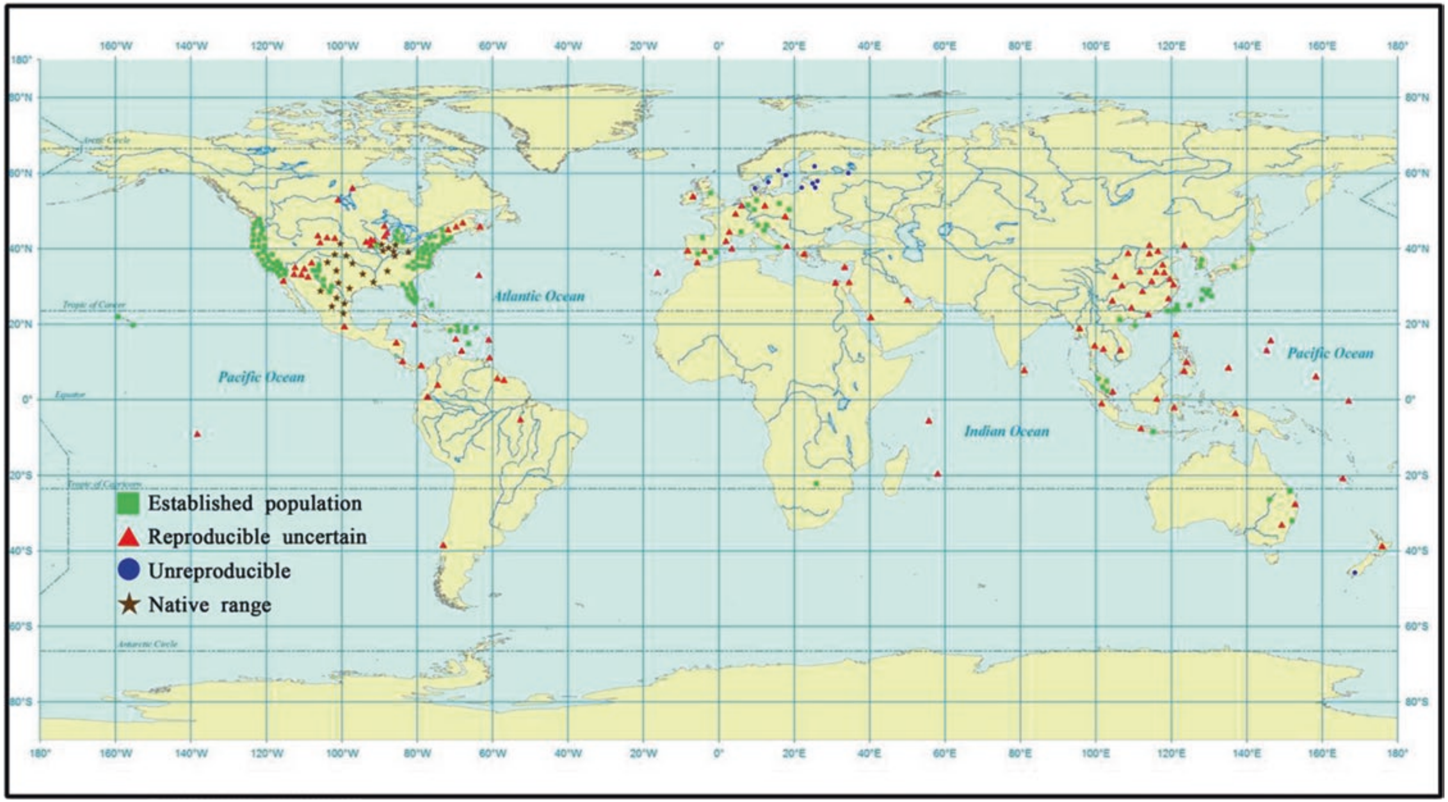

Fig. 21.2 The distribution of red-eared slider around the world

where it poses a threat to native biodiversity (Shi et al. 2009). In order to clarify the population status, mechanisms of ecological adaptation, impacts on the environments and society, and to develop control measures in China, a series of studies on the invasion ecology of the exotic red-eared slider have been conducted since 2008 by the Chelonian conservation biology research group of Hainan Normal University under the leadership of professor Haitao Shi.

21.2 Occurrence Status

We investigated the geographical distributions of the red-eared turtles across the whole China for the purpose of understanding the invasion status of this species. The survey showed that there was not only a huge consumer demand but also a very large scale of slider breeding nationwide. Moreover, this turtle has a wider field distribution range than any native turtles in China. Thus, the question is why the exotic species can be so successful.

To answer this question, we chose four typical study sites that differ in latitude, climate zone, habitat type and altitude: Nanjing section of Yangtze River (NYR), Gutian Nature Reserve (GNR), Haikou section of Nandu River (HNR), and Qionghai section of Wanquan River (QWR) (Table 21.1).

21.2.1 Distribution

The red-eared turtle has been farmed in 17 provinces of China, most of which locate in eastern China (Liu et al. 2011; Fig. 21.3). At present, some provinces such as Jiangsu, Zhejiang, Hunan, Guangdong and Hainan have a large number of farms.

A large number of slider trade markets have been established throughout the country, even in some remote districts such as Xinjiang, Tibet, Heilongjiang and Yongxing Island, South China Sea. The trade scale is extremely huge, for example, the average daily traded amount reaches tens of thousands of individuals both in Huadiwan and Qingping pet markets, Guangzhou city. Furthermore, the slider was also found in the Free Life Pond of 51 temples in 18 provinces (Liu et al. 2011; Fig. 21.3).

Table 21.1 The general situation of study sites

Study sites	Geographical coordinates	Climate zone	Habitat type	Altitude
NYR	N 32°04′, E 118°42′	Northern subtropics	River	5 m
GNR	N 23°06′, E 114°47′	Southern subtropics	Stream	250 m
HNR	N 20°04′, E 110°37′	Tropic	Estuary	6 m
QWR	N 19°15′, E 110°27′	Tropic	River	6 m

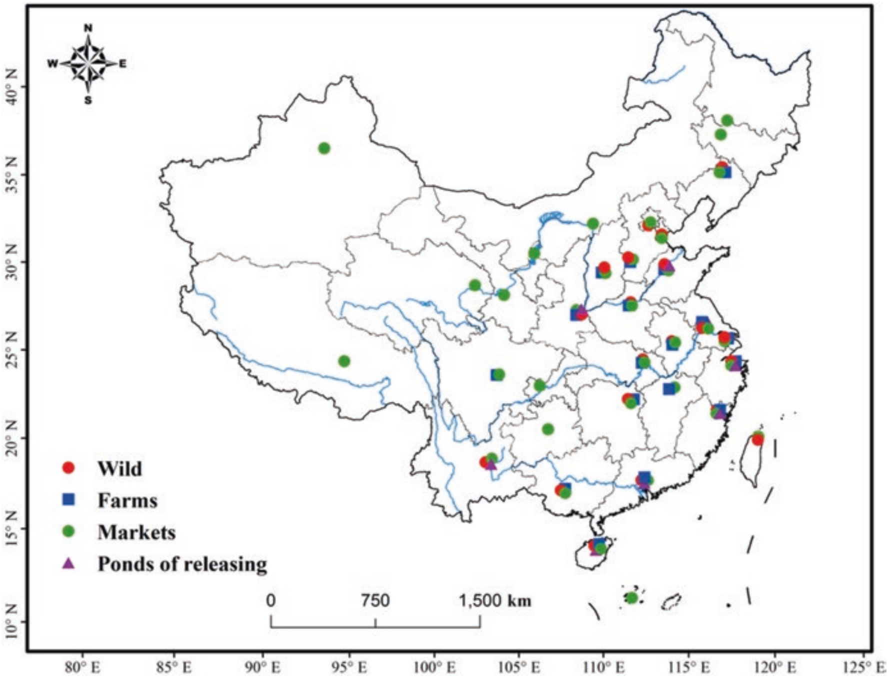

Fig. 21.3 Distribution of the red-eared slider in China

According to the field survey, the red-eared turtle is widely distributed in 22 provinces of China, covering an area of more than 3 million km^2. Its distribution areas are mainly in the central and southern China (Liu et al. 2011; Fig. 21.3).

21.2.2 Dispersal Pathway

In China, the red-eared slider for trade purpose came from both import and farming, of which only a small part were eaten as food and most were released into the wild by people, including religious release, faulty release, and abandoned pets. In addition, some captive turtles could escape to the wild (Ma 2013; Fig. 21.4).

Religious release is the main reason for the wide distribution of this turtle in the wild in China. The people in religious conscience believe that release animals can rescue these creatures, but most of them adopted the wrong way that would probably result in biological invasions (Agoramoorthy and Hsu 2007; Liu et al. 2013). The religious people usually did not consider whether these animals are invasive species.

In addition, the turtle species is considered as an auspicious and long-lived animal in Chinese traditional culture, and the ritual freeing of turtles will bring good

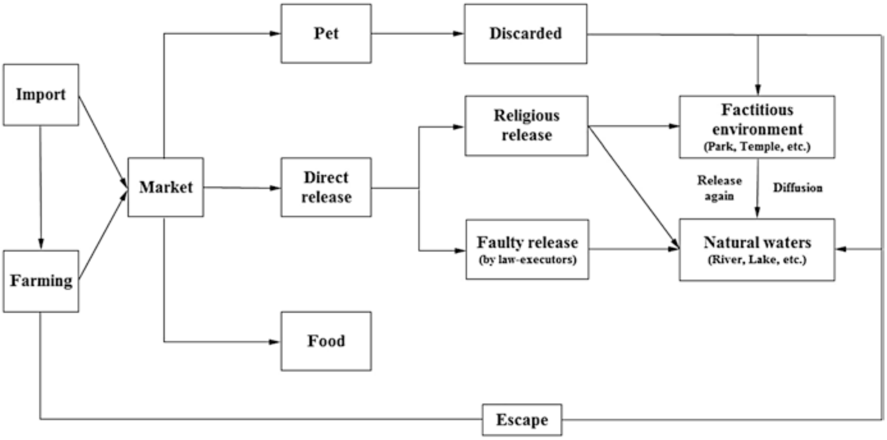

Fig. 21.4 Diffused pathway of red-eared slider in China

luck to the people who are praying. The red-eared slider is the most common turtle species in trade markets, and its price is far lower than native turtles. Thus, the religious people prefer to release the red-eared turtles into the wild.

There two kinds of religious releases: group release and individual release. The group releases are normally sponsored by religious associations and temples, performed by holding a grand ceremony, and have a low frequency. For this type of release, usually a huge number of turtles are released in a single activity, and Chinese characters and/or religious patterns are not carved on shells. By contrast, the individual releases are an unorganized behavior performed by the believers, with no or only a simple ceremony being held, and at a higher frequency; a few of animals are released in a single activity, and usually carved with Chinese characters (e.g. Buddha, name, date of freeing, etc.) and/or religious patterns on shells. The turtles carved with symbols would be able to live longer than those turtles without symbols (Ma 2013). This is because turtles with symbols would not be captured by local fishermen who believe that selling or killing such turtles is an infelicitous thing. Therefore, ritual release of animals may threaten ecological safety.

Furthermore, pet abandoned, faulty release and farming escape are the important ways of red-eared turtles entering into wild environments. Although the slider hatchlings look gentle and lovely, the aggressiveness will increase gradually as the body size increases. For this reason, most pet owners choose to discard them after the slider becomes a large one. In some cases, policemen treat the confiscate exotic species (such as *T. s. elegans*, *Chelydra serpentina*) as native species and release them into the wild, because they lack related professional knowledge. In addition, as turtle farms are normally built in the wild and there are often a large number of mature red-eared turtles in breeding pools (where gravid females are present), some turtles probably escape and establish the natural breeding populations in the wild.

It is a remarkable fact that artificial habitats (such as park, temple) play a role as transit station in promoting the red-eared turtles spread into the wild. In China, we can find the free-living sliders in almost all pools in park and temples (Liu et al. 2011). Ponds are used for short storage of turtles used for releasing into the wild. There is a common phenomenon; the administrators of park and temple would release lots of sliders into the natural waters at a nonscheduled way. For example, in 2009, more than 100 sliders from the pond of Boao monastery were released into the Wanquan River by the local monks in Qionghai, Hainan Island. After releasing, the turtles probably disperse into the neighboring wild regions.

Taken together, in China, a large number of imports and the breeding of red-eared turtles have made this exotic species become the most common turtles in trade markets. Such a case appears to have promoted the conscious or unconscious releases of this turtle into the wild by religionists, law-executors and pet owners, which poses a great threat to the local biodiversity.

21.2.3 Population Structure

The red-eared slider could be found in all of the research areas where it is a dominant species in local communities. The population structure of sliders is seriously unbalanced in the wild, such as the heavy sex ratio deviation and a very low percentage of juveniles. It had a clear female-biased sex ratio in the Yangtze River, Wanquan River and Gutian Nature Reserve. In Nandu River, however, the sex ratio was slightly male biased.

This pattern is mainly associated with the releasing of a large number of captive sliders into the wild (Ma 2013; Wang 2013). In turtle farms, some warming measures were taken to increase hatching rate and shorten incubation period. As a result, the female would hatch at a higher rate than the male under a relatively high incubation temperature, because the slider is a temperature-dependent sex determination species (Willingham 2005). Thus, more female sliders derived from farms were released into the wild, and caused a clear female-biased sex ratio. However, a slightly male-biased sex ratio may be due to a relatively high natural mortality rate of female sliders at the surveyed site in Nandu River, where there is estuary brackish water environment (Yang 2014). In addition, adult sliders were sold much more than juveniles in markets due to high profits. Consequently, some commercial aspects in the farming of sliders, together with many “creature-releasing” activities, have a strong impact on the population structure of feral sliders.

The relative density of red-eared turtles is much higher than that of native turtles, ranging from 0.0015 to 1.25 per trap day. The proportion of sliders in all of freshwater turtles is extremely high. The maximum is 98.7% in Nandu River, and the minimum is 60.2% in Yangtze River. In fact, the degree of endangered of Chinese native turtles is far beyond our imagination (Table 21.2).

Table 21.2 The collected turtles in all of study sites

Species name	Origin	Total numbers	Percentage
Red-eared turtle *T. s. elegans*	Exotic	302	83.0%
Chinese pond turtle *Mauremys reevesii*	Native	36	9.9%
Chinese stripe-necked turtle *Mauremys sinensis*	Native	18	4.9%
Common snapping turtle *Chelydra serpentina*	Exotic	3	0.8%
Beal's eyed turtle *Sacalia bealei*	Native	3	0.8%
Southeast Asian box turtle *Cuora amboinensis*	Exotic	2	0.5%

Table 21.3 The home range of red-eared slider in different studies

Locality	Habitat	Sample size	Females' home range (hm^2)	Males' home range (hm^2)	Total home range (hm^2)	Study period (month)	References
QWR	River	19 F, 8 M	11.39	1.83	8.15	21	Ma et al. (2013)
GNR	Stream	13 F, 8 M	2.16	10.26	5.25	17	Zhou et al. (2013)
HNR	Estuary	5 F, 7 M	3.26	4.23	3.83	10	Yang and Shi (2014)
South Carolina, USA	Lake	7 F, 9 M	36.53	103.53	74.22	16	Schubauer et al. (1990)

Note: home range is an area that exploited by animals for feeding, mating, reproducing and other normal activities

21.3 Mechanisms of Population Establishment and Spread

21.3.1 Ecological Adaptability

21.3.1.1 Home Range

The home range area of sliders in China (about 3–8 hm^2) was much smaller than that in its native range (about 74 hm^2) (Table 21.3). This suggests that the sliders do not move for a long distance after introductions, possibly due to the plenitudinous availability of food, stabile habitats and suitable climate in China. In other words, the slider can grow and reproduce safely within a limited range. Such a life style would help reduce the potential risk from predators, and also reduce the energy cost that would otherwise be required to maintain population fitness after spreading to a larger range.

Population sex ratio and the type of habitat may be the main factors affecting the home range of red-eared turtles in China. The home range of males was significantly wider than that of females in the HNR (Yang and Shi 2014) and the GNR (Zhou et al. 2013). On the contrary, the situation was reversed in the QWR (Ma et al. 2013). Such a difference was attributed to the difference in sex ratio and habitat between the two localities. In the populations with male-biased sex ratio, males need a larger range of activity for reproduction purpose as compared with the males in female-biased populations. As a result, the energy that males invest for mating may be greatly affected by sex ratio (Thomas et al. 1999), as reflected in the distance of males' movement (Ma et al. 2013). Similarly, females need a larger range of activity in a river habitat than in a stream habitat. In the river habitat, water level changes quickly and drastically, thus female turtles have to move a longer distance for nesting (Tucker and Moll 1997; Ma 2013).

Besides, the home range of red-eared slider has also been compared with native turtles in China, such as *Mauremys sinensis*. The red-eared slider and *M. sinensis* were found to have a similar home range area, however, the former had a significantly larger intraspecific home range overlap than the latter (Ma et al. 2013). The interspecific home range overlap degree between the two species was as large as 0.20 ± 0.02[1] (Ma et al. 2013). The results suggest that the red-eared turtles are less restricted by spatial factors, and can adapt well to living in groups with high density. This feature was also observed in populations in other countries. In Savannah River in South Carolina, USA, for example, the population density of red-eared slider could reach a maximum of 1000 individuals per hm^2 (Gibbons and Avery 1990a). However, *M. sinensis* showed an obviously dispersed spatial pattern. Therefore, there is a potential interspecific competition between the sliders and native turtles on space resources, and *M. sinensis* may need more space for population growth.

21.3.1.2 Habitat

The red-eared slider can adapt to different types of habitats very well, including rivers (Liu 2011; Wang 2013), streams (Hu 2012), and even in brackish water (Salinity 5.3–14.6‰) environments (Liu 2011; Yang 2014). Studies on habitat selection and microhabitat utilization showed that this species prefers to live in various freshwater areas, in particular those abundant with aquatic animals and plants, moderate canopy density, suitable concealment and basking places, and still or slow flowing shallow water (Liu 2011; Hu 2012). It also prefers to live in brackish water habitats which are abundant with algae, moderate canopy density, high hidden degree, and near-shore shallow water (Yang 2014).

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[1] The home range overlap degree was measured by overlap index that range is between zero and one. And a higher value means more overlap in home range.

Furthermore, Wang (2013) conducted an extensive comparative study on the habitat selection between the sliders and *M. sinensis* in the QWR. The results showed that both species favored the habitat type of riparian vegetation. The Levin's niche breadth of the sliders is higher than that of *M. sinensis*, which suggests that the sliders have a higher adaptability to new environments. There are very high interspecific overlaps between two species for all variables of microhabitats. These imply a possibility of competition between the invasive turtle and the native *M. sinensis* over spatial resources.

In addition, Chen et al. (2014) evaluated the habitat suitability for the red-eared slider by the "3S technology" (GPS, RS, GIS) in the QWR. The result showed that elevation, distance to water and human disturbance have a significantly influence on the habitat selection of slides. The red-eared slider preferred to the areas with an altitude of 0-15 m, a distance of 200-400 m from human disturbance to water, and a distance below 100 m from artificial forest to water. According Markov prediction model, the suitable habitat of red-eared slider will continue to increase in the next 30 years, which suggests that the sliders will spread easily and native turtles will be highly effected (Chen 2014).

21.3.1.3 Diets

The red-eared slider is an typical opportunistic omnivorous animal who consumes a wide variety of invertebrates (shellfishes, snails, shrimps, crabs, insects), vertebrates (fishes, frogs, lizards, snakes, birds, rodents), and plants (algae, ferns, seed plants) in China. Of these foods, 41 species belong to animals in 35 genera of 29 families, and 49 species belong to higher plants in 43 genera of 27 families (Table 21.4). In fact, the food varieties are far more than the above mentioned, as some prey diets could not be detected and identified by stomach flushing and fecal analysis techniques due to the strong digestion ability of sliders.

There was a significant difference in the ratio of animals and plant materials eaten by the red-eared turtle. Sliders consumed more animals than plants in the QWR and HNR (Liu 2011; Wang et al. 2013; Yang 2014), where mollusks and fishes, and fishes, shrimps and crabs are the major food, respectively. However, they consumed more plants than animals in the GNR (Hu 2012), where ferns, gramineous grass, and commelinaceae are the major food.

Furthermore, the red-eared slider has a diet difference with the one of native turtle species (*M. sinensis*; Wang et al. 2013). For example, in QWR, the slider takes native snails and fishes as the most important food items, while grass makes up the majority of the diet of *M. sinensis*. For this reason, a large number of indigenous aquatic species are eaten by the slider, which harms the native ecosystems directly. It may also play a negative role in the process of community succession, by changing the structure of local communities. As a result, it promotes biological invasions of other alien species, such as apple snail (*Ampullaria gigas*) and water hyacinth (*Eichhornia crassipes*).

Table 21.4 The diet composition of food items in stomach and feces of red-eared slider in different study area in China

Food items							Sample size	Study area	References
Kingdom	Phylum	Class	Order	Family	Genus	Species			
Animalia	Mollusca	Lamellibranchia	Mytiloida	Mytilidae	*Modiolus*	*M. modiolus*	202 (127 F, 33 M, 42 J)	QWR	Liu (2011) and Wang (2013)
						M. philippinarum			
			Unionoida	Unionidae	*Anodonta*	*A. globosula*			
						A. woodiana			
			Veneroida	Corbiculidae	*Corbicula*	*C. fluminea*			
						C. nitens			
		Gastropoda	Mesogastropoda	Thiaridae	*Melaniodes*	*M. tuberculata*			
					Sermyla	*S. riqueti*			
				Melanidae	*Brotia*	*B. swinhoei*			
				Viviparidae	*Angulyagra*	*A. polyzonata*			
					Bellamya	*B. purificata*			
				Ampullariidae	*Ampullaria*	*A. gigas*[a]			
	Arthropoda	Crustacea	Decapoda	Palaemonidae	*Macrobrachium*	*M. hainanense*			
				Potamidae	UN				
		Insecta	UN						
	Chordata	Osteichthyes	UN						
		Aves	UN						
		Mammalia	Rodentia	UN					

(continued)

Table 21.4 (continued)

Food items							Sample size	Study area	References
Kingdom	Phylum	Class	Order	Family	Genus	Species			
Plantae	Angiospermae	Monocotyledoneae	Farinosae	Pontederiaceae	*Eichhornia*	*E. crassipes*[a]			
				Commelinaceae	*Commelina*	*C. communis*			
			Arales	Araceae	*Pistia*	*P. stratiotes*			
					Typhonium	*T. giganteum*			
				Lemnaceae	*Lemna*	*L. minor*			
			Graminales	Gramineae	*Phragmites*	*P. karka*			
					Bambusa	*B. blumeana*			
					Eleusine	*E. indica*			
		Dicotyledoneae	Centrospermae	Amaranthaceae	*Alternanthera*	*A. philoxeroides*[a]			
						A. pungens[a]			
			Urticales	Moraceae	*Ficus*	*F. heterophylla*			
			Tubiflorae	Convolvulaceae	*Ipomoea*	*I. aquatica*			
			Myrtiflorae	Onagraceae	*Ludwigia*	*L. octovalvis*			
			Euphorbiales	Euphorbiaceae	*Phyllanthus*	*P. niruri*			
			Rosales	Leguminosae	*Pongamia*	*P. pinnata*			
			Umbelliflorae	Umbelliferae	UN				

Animalia	Mollusca	Lamellibranchia	Veneroida	Veneridae	*Paphia*	*P. amabilis*	80 (26 F, 41 M, 13 J)	HNR	Liu (2011) and Yang (2014)
		Gastropoda	Mesogastropoda	Potamodidae	*Cerithidea*	*C. ornata*			
				Viviparidae	*Bellamya*	*B. purificata*			
					Angulyagra	*A. polyzonata*			
				Thiaridae	*Melaniodes*	*M. tuberculata*			
	Arthropoda	Crustacea	Decapoda	Palaemonidae	*Macrobrachium*	*M. hainanense*			
				Penaeidae	*Penaeus*	*P. monodon*			
						P. merguiensis			
	Chordata	Osteichthyes	Perciformes	Cichidae	*Tilapia*	*T. nilotica*[a]			
						T. mossabica[a]			
				Gobiidae	*Periophthalmus*	*P. cantonensis*			
		Amphibia	Anura	Bufonidae	*Bufo*	*B. melanostictus*			
		Reptilia	Squamata	Scincidae	*Mabuya*	*M. multifasciata*			
				Colubridae	*Xenochrophis*	*X. piscator*			
		Aves	UN						
		Mammalia	Rodentia	UN					

(continued)

Table 21.4 (continued)

Food items							Sample size	Study area	References
Kingdom	Phylum	Class	Order	Family	Genus	Species			
Plantae	Chlorophyta	Chlorophyceae	Cladophorales	Cladophoraceae	*Cladophora*	UN			
	Angiospermae	Monocotyledoneae	Farinosae	Pontederiaceae	*Monochoria*	*M. vaginalis*			
			Liliflorae	Liliaceae	*Asparagus*	*A. schoberioides*			
			Graminales	Gramineae	*Phragmites*	*P. australis*			
					Eleusine	*E. indica*			
					Setaria	*S. viridis*			
		Dicotyledoneae	Tubiflorae	Convolvulaceae	*Ipomoea*	*I. pes-caprae*			
				Verbenaceae	*Lantana*	*L. camara*[a]			
					Clerodendrum	*C. inerme*			
			Centrospermae	Aizoaceae	*Sesuvium*	*S. portulacastrum*			
			Campanulales	Compositae	*Silybum*	*S. marianum*			
					Praxelis	*P. clematidea*[a]			
					Parthenium	*P. hysterophorus*[a]			
			Rosales	Leguminosae	*Cassia*	*C. surattensis*			
					Pongamia	*P. pinnata*			
			Rubiales	Rubiaceae	*Borreria*	*B. stricta*			
					Paederia	*P. scandens*			
			Verticillatae	Casuarinaceae	*Casuarina*	*C. equisetifolia*			

Animalia	Mollusca	Gastropoda	Neogastropoda	Melongenidae	*Hemifusus*	*H. tuba*	84 (57 F, 25 M, 2 J)	GNR	Hu (2012)
	Arthropoda	Insecta	Coleoptera	Elateridae	*Campsosternus*	*C. auratus*			
				Lucanidae	*Neolucanus*	*N. sinicus*			
				Rutelidae	*Mimela*	UN			
			Hemiptera	Coreidae	*Homoeocerus*	*H. marginellus*			
			Odonata	Libellulidae	UN				
			Orthoptera	Pyrgomorphidae	*Atractomorpha*	UN			
	Chordata	Osteichthyes	Cypriniformes	Cyprinidae	*Carassius*	*C. auratus*			
					Puntius	*P. semifasciolatus*			
					Cyprinus	*C. carpio*			
			Perciformes	Channidae	*Channa*	*C. maculata*			
		Amphibia	Anura	Bufonidae	*Bufo*	*B. melanostictus*			
				Ranidae	*Hylarana*	*H. guentheri*			
						H. latouchii			
		Reptilia	Squamata	Scincidae	*Sphenomorphus*	*S. indicus*			
				Colubridae	*Xenochrophis*	*X. piscator*			
					Sinonatrix	*S. percarinata*			

(continued)

Table 21.4 (continued)

Food items							Sample size	Study area	References
Kingdom	Phylum	Class	Order	Family	Genus	Species			
Plantae	Pteridophyta	Filicopsida	Eufilicales	Athyriaceae	*Callipteris*	*C. esculenta*			
				Lygodiaceae	*Lygodium*	*L. scandens*			
	Angiospermae	Monocotyledoneae	Farinosae	Commelinaceae	*Commelina*	*C. communis*			
			Graminales	Gramineae	*Leersia*	*L. hexandra*			
					Phragmites	*P. australis*			
					Miscanthus	*M. floridulus*			
					Bambusa	UN			
			Cyperales	Cyperaceae	*Cyperus*	UN			
		Dicotyledoneae	Centrospermae	Amaranthaceae	*Alternanthera*	*A. philoxeroides*[a]			
					Celosia	*C. argentea*			
			Umbelliflorae	Umbelliferae	*Oenanthe*	*O. rosthornii*			
					Hydrocotyle	*H. sibthorpioides*			
			Myrtiflorae	Onagraceae	*Ludwigia*	*L. hyssopifolia*			
			Geraniales	Oxalidaceae	*Oxalis*	*O. corniculata*			
			Campanulales	Compositae	*Scorzonera*	*S. albicaulis*			
			Parietales	Theaceae	*Eurya*	*E. nitida*			
			Urticales	Moraceae	*Ficus*	*F. variolosa*			
			Tubiflorae	Acanthaceae	*Hygrophila*	*H. salicifolia*			
			Rosales	Leguminosae	*Caesalpinia*	*C. crista*			
			Polygonales	Polygonaceae	*Polygonum*	*P. lapathifolium*			
			Primulales	Primulaceae	*Lysimachia*	*L. fortunei*			

[a] Exotic species; *UN* Unrecognized, *F* Females, *M* Males, *J* Juveniles

Table 21.5 The basic data of reproduction effort of red-eared slider in China

Turtle No.	Laying date	Clutch size	Hatchlings	Hatchability (%)	Incubation period (days)	Study area
174	2012.04.25	11	0	0	–	QWR
188	2012.04.27	11	0	0	–	
186	2012.04.28	10	0	0	–	
197	2012.05.12	14	4	28.6	74	
196	2012.05.14	13	0	0	–	
194	2012.05.14	13	2	15.4	72	
188	2012.05.18	12	2	16.7	78	
194	2012.06.06	15	3	20.0	57	
83	2013.05.02	11	5	45.5	64	HNR
102	2013.05.05	10	1	10.0	56	
102	2013.05.26	8	5	62.5	61	
104	2013.05.14	12	4	33.3	68	
115	2013.05.27	14	0	0	–	

21.3.1.4 Reproduction

The red-eared turtles can successful reproduce in the wild in southern China. Natural nests and hatching turtles were both found in the QWR and HNR (Li 2013; Yang 2014; Table 21.5). There was no direct evidence that it can reproduce in the GNR. But such an possibility exists, because in this locality both natural nests and juveniles less than one year old were found, and hatching turtles emerged from the artificial nests in the wild (Zhou 2013).

In Hainan Island, Li (2013) and Yang (2014) conducted an extensive study on the reproductive ecology of red-eared slider in the QWR and HNR, respectively. In general, the reproduction of red-eared slider starts in February and lasts to the end of August. The females lay their eggs predominantly from mid-April to mid-June, with the peak occurring from late April to late May. They prefer to nests and lay eggs in such places that are located on gentle sunny slope, near shore, abundant with vegetation, with the soil being soft, wet and weakly acidic but not muddy.

Furthermore, we also detected the number and size of follicles of feral female sliders by ultrasonic technique in the QWR from October 2011 to September 2012 (Li et al. 2013; Fig. 21.5). The data showed that the females had a breeding frequency of 2.48 clutches per year, which suggests that sliders have a great reproductive ability. Such a reproductivity probably should contribute to its wide spread and population development in the wild.

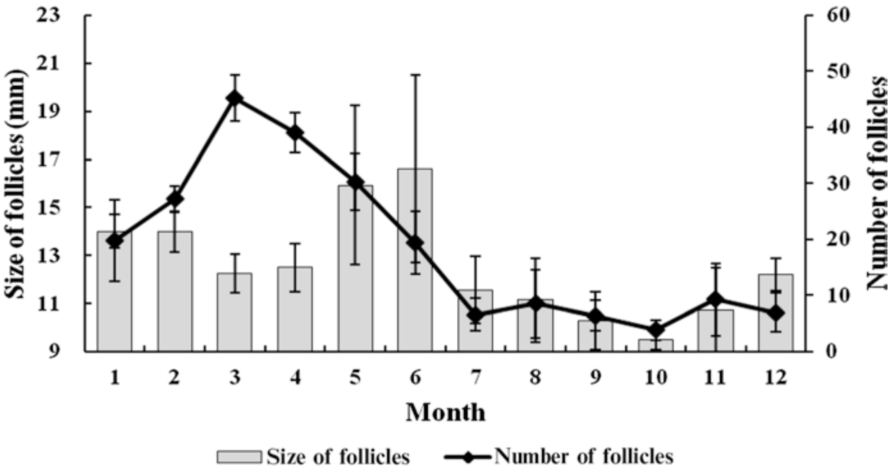

Fig. 21.5 The mean size and number of follicles per month of feral female sliders in the QWR

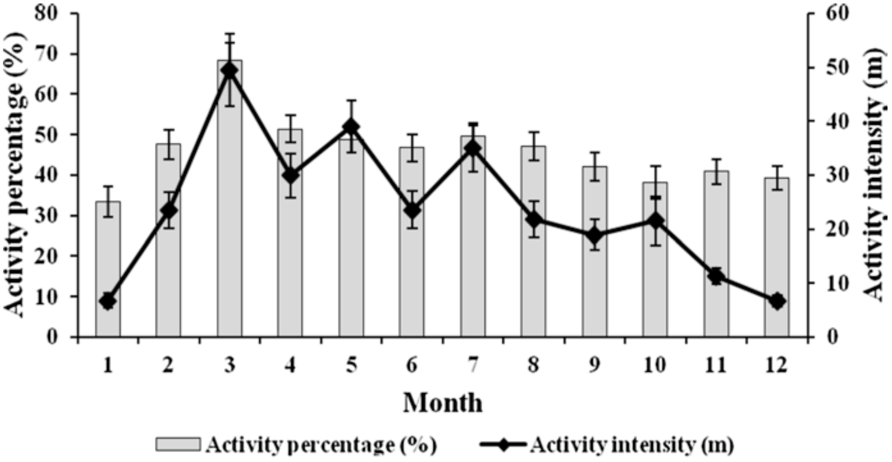

Fig. 21.6 Annual activity rhythm of red-eared slider

21.3.1.5 Behavior

Ma (2013) conducted an extensive study on the activity rhythm of the red-eared slider in the QWR from November 2011 to October 2012. The sliders are active in every month, and they do not enter hibernation and aestivation (Fig. 21.6). They are primarily diurnal exhibiting a unimodal pattern in daily activity (Fig. 21.7). Overall, the red-eared slider performs very well under local climate conditions, because the temperature is suitable (The annual average temperature 28.1°C was just within the most suitable activity temperature range between 28°C and 29°C for sliders).

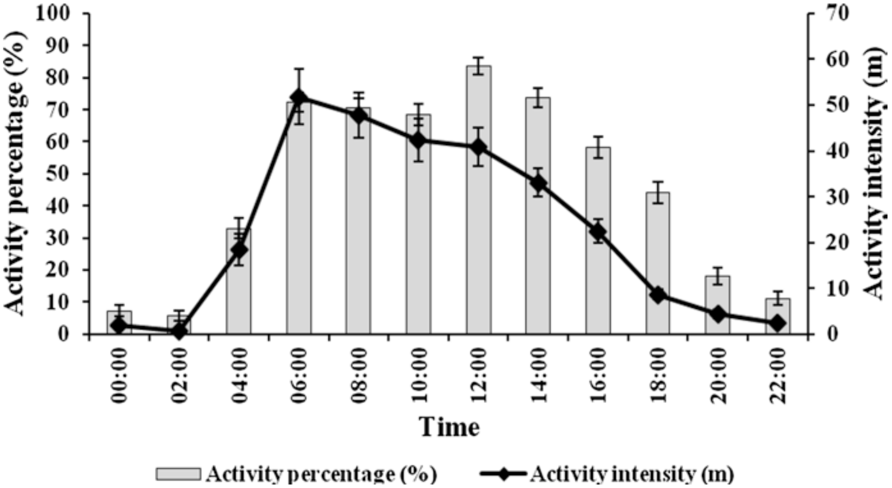

Fig. 21.7 Daily activity rhythm of red-eared slider

Temperature may be an indispensable reason why this species could make a successful invasion in Hainan Island.

In the daily activity of sliders, the basking behavior is a very important thermoregulation process. For this reason, we studied the basking habit in the QWR (Ma 2013). Basking time showed a clear unimodal pattern, with a peak in 11:00–13:00. The body surface temperature was significantly higher during periods of basking in comparison to periods of non-basking. During basking, the temperature of carapace surface increased at a rate of 0.18°C/min, and as the basking end it reached approximately 36°C. The results suggest that sliders have a thermal preference, and they could fully and effectively exploit the thermal heterogeneity of surrounding environments.

21.3.1.6 Physiology

In laboratories, the red-eared turtle has a strong tolerance to environmental stresses, by giving a series of physiological and biochemical responses to harsh conditions. As compared with the native species *M. sinensis*, embryos of sliders had a higher survival on ammonium nitrate and acid stresses (Fu et al. 2012a, b, 2013; Li et al. 2014b), as do the juveniles on the chronic nitrite stress (Zhang et al. 2011). This suggests that, when facing ambient stress, the slider is probably able to improve its anti-oxidant ability and nonspecific immunity function by certain approaches, such as increasing the number of haemocyte and changing the morphology of red blood cell. In addition, the slider also has stronger starvation endurance than *M. sinensis* (Wei et al. 2012). This suggests that the slider may conserve food much efficiently and would reduce basal metabolic rate in the case of food shortage.

Moreover, the red-eared slider has a high adaptability to salinity stress. They are able to survive over three months in the brackish water with a salinity below 15‰ (Zhang 2014). This is because sliders can increase blood osmotic pressure by balancing the entry of NaCl with the secretion of aldosterone decreased, and by accumulating blood urea for osmoregulation effectors (Hong et al. 2014; Zhang et al. 2014). Besides, they also improved the metabolic level by increasing blood-sugar content and enzymatic activities to provide energy required for resisting salinity stress (Shu et al. 2012).

21.3.2 The Role of Local Communities Impedance

No native predators that hunt the adult, juvenile, or hatching sliders have been found in China. But some small animals can destroy the eggs of sliders, who are called nest predators. Nest predation is a primary cause of partial reproductive failure of sliders in the wild. This is the only spontaneous resistance force so far discovered that are exerted by native biological communities. Nest predation should have prevented the population growth of sliders, and as a consequence, have repressed their invasions to a certain extent.

For example, in Hainan island, eggs in the nest were primarily eaten by Taiwanese kukrisnakes (*Oligodon formosanus*) and ants (*Polyrhachis dives* and *Pheidole indica*), and the predation rates in the QWR and HNR reached 33.3% and 22.0%, respectively (Li 2013; Yang 2014). In the GNR, nearly all of nest predators were ants, including *Pheidole* sp., *Pheidologeton* sp., *Camponotus albosparsus*, *Camponotus nicobaresis*, *Plagiolepis mthneyi*, *Tapinoma melanocephalum*, *Paratrechina bourbonica*, which made an overall predation rate of approximately 65.6% (Zhou 2013).

Furthermore, Li et al. (2014a) investigated the type of nest predators, predation rates and the breeding success of the red-eared slider by simulating artificial nests at the Diaoluoshan village, Diaoluo Nature Reserve in Hainan island, which is the typical habitat of Indo-Chinese box turtle (*Cuora galbinifrons*). The result showed that 66.7% of nests were depredated and small mammals (51.3%, such as *Niviventer fulvescens* and *Tupaia belangeri*) and ants (28.8%) were the main predators.

In addition, the predation rates in the nature reserve (e.g. Gutian Nature Reserve & Diaoluo Nature Reserve) were higher than in the areas more disturbed by human activities, such as the QWR and HNR that locate on urban peripheral regions. This suggests that the community with higher biodiversity and more stable structure might have a relatively stronger resistance to alien species. In other words, the red-eared turtle is probably ready to invade such areas that suffer from habitat loss, degradation, and fragmentation.

21.4 Impacts

21.4.1 Impacts on Native Turtle Species

In order to get a scientific evaluation of the red-eared slider's impacts on native turtle species, we have conducted a series of comparative studies on this slider and the representative of indigenous species. In China, the red-eared slider lives with at least 14 native turtle species (e.g. *Mauremys sinensis*, *M. reevesii*, *M. mutica*, etc.) which account for 50% of total freshwater turtle species. Living in the same habitats have resulted in interspecific competition, including exploitation competition, interference competition and apparent competition (Shi et al. 2009; Ma et al. 2013; Wang 2013).

Firstly, the red-eared slider with a huge number of individuals would consume and occupy a lot of resources, such as food, basking sites, and nesting sites, which may induce intensive exploitation competition with native turtles. Such a type of competition has been found between the slider and *M. sinensis*. The two species have a sympatric distribution, and exploit the same spatial and food resources (Ma 2013; Wang 2013). As the sliders are more agile, aggressive and efficient in competition for food than *M. sinensis*, the native species obviously stands in the disadvantaged position in the competition (Zhao et al. 2013).

Secondly, the red-eared slider attacks the native turtles through biting, climbing, swiping, and pushing, and disturbing the courtship and mating activities of native turtles. Sometimes, sliders even mate with native turtles and produce hybrids, thereby negatively affecting reproduction and population development of the natives. For example, we found the hybrid of the red-eared slider and Chinese stripe-necked turtle in November 2007 in the Huadiwan market, Guangzhou (Shi et al. 2009; Fig. 21.8). Thus, the sliders might have polluted the genetic constitution of feral populations of native turtles through reproductive interference and hybridization (Ma et al. 2015).

Fig. 21.8 The hybrid of *T. s. elegans* and *M. sinensis* (Photo by Shi-Ping Gong)
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Thirdly, apparent competition occurs when both native turtles and the sliders are captured by human. As the wild population of feral sliders grows, the number of turtle-capturing fishermen (including professional turtle hunters) is increasing (Ma 2013; Wang 2013). However, hunters prefer to capture the native turtles, which may have the higher price of aboriginal species (e.g. *M. sinensis* is about 200 RMB/kg vs. 80 RMB/kg of feral *T. s. elegans*). This phenomenon raises the risk for native turtles being captured and thus poses a serious threat to the indigenous animals in China.

In conclusion, if the slider population grows, then the fitness of vulnerable native turtles may be reduced due to the lack of available spatial and food resources. As a result, native species might be squeezed out of suitable habitats over time and finally extinct.

21.4.2 Impacts on Human Health

The red-eared turtle may have a potential risk on human health by transmitting Salmonellosis, one of the important anthropozoonosis caused by *Salmonella*. In human beings, especially children and people with weak immune systems, *Salmonella* causes diarrhoea, cramps, fever, and nausea, and can lead to more serious complications such as blood poisoning, meningitis, or even death (Tauxe et al. 1985; Centers for Disease Control Prevention 2007). There are about 130 million people each year infected by this pathogen and suffer from enterogastritis in the world (Pang et al. 1995). In the United States, Salmonellosis is a significant public health concern, which causes about 1.4 million illnesses and 400 deaths each year (Voetsch et al. 2004). Of them, large numbers of *Salmonella* infections have been traced back to pet terrapins, most of which were *T. s. elegans* (Ramsay et al. 2007).

In China, we found that the feral sliders have a high rate of carrying pathogenic *Salmonella*. Shen et al. (2011) detected the pathogen in 68 red-eared turtle individuals from Nandu River, Wanquan River and Haikou East Lake in Hainan Island. They found that 54.4% of these turtles carried *Salmonella*, and the rate in Nandu River, Wanquan River and Haikou East Lake reached 53.85%, 58.82% and 50%, respectively. The indentified *Salmonella* serotypes included *S*. Litchfiled, *S*. Chailey, *S*. Senftenberg, *S*. Newport, *S*. Tshiongwe, and *S*. Stanley. The rate did not vary significantly with sex, but it was significantly higher in juveniles than in adults (Shen 2011). Furthermore, *Salmonella* Pomona, a highly pathogenic serotype, was isolated from free-living exotic red-eared sliders in the wild in China for the first time (Gong et al. 2014). In the samples collected from the Gutian Nature Reserve, Guangdong province, the carrying rate of *S*. Pomona was 39% ($n = 41$), with 40% ($n = 25$) in juveniles and 38% ($n = 16$) in adult turtles.

Overall, the red-eared slider is promoting the spread of *Salmonella* in human and aquatic ecosystems. People have the possibility of being infected with this pathogen by direct or indirect contacts with red-eared turtles (Fig. 21.9). Therefore, lots of red-eared sliders in pet/food markets and in the wild are potentially huge sources of infection for *Salmonella*, posing a great potential threat to public health and ecosystems of China by transmitting Salmonellosis (Gong et al. 2014).

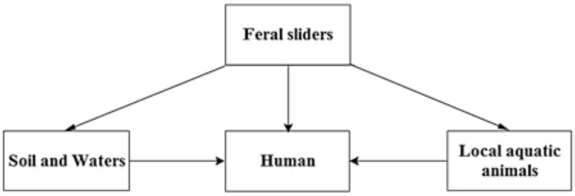

Fig. 21.9 The route of transmission in Salmonellosis via *T. s. elegans*

21.5 Management and Control

In the context of threatened and endangered species, introductions of alien turtle species should always be stiffly banned and governments should prevent the importation of freshwater turtles (Cadi and Joly 2003). In face of the serious threats from the exotic red-eared turtle, we need to take some measures to prevent or reduce such damages.

Firstly, enact relevant laws which ban the import of *T. s. elegans*. In 1975, the U.S. Food and Drug Administration prohibited the sale of turtles below 4 in. (ca. 10 cm) in carapace length in the United States and Canada because they could transmit salmonellosis by carrying bacterial pathogens *Salmonella* and *Arizona* (Bringsøe 2006). In 1997, the 16-member European Union banned the import of red-eared sliders on the grounds that they were having a deleterious effect on the indigenous European pond terrapin (*E. orbicularis*) (Ramsay et al. 2007). In 2001, the Ministry of Environment of South Korea banned the import of red-eared sliders due to potential ecosystem disturbance (Lee and Park 2010). However, currently in China, there have not been any laws and regulations to ban the import of *T. s. elegans*, hence about 8 million sliders still are imported into the country through various ways every year (Shi et al. 2009). Therefore, legislation should be enacted by the Chinese government as soon as possible.

Secondly, make a standardized management of turtle farms and trade markets. In the past, people in China always thought that the commercial production of turtles would greatly reduce the demands and consumption of wild native turtles. Actually, however, the massive culture of sliders could not alleviate the survival pressure posed on the wild natives. It appears difficult to change people's preference for eating wildlife, but instead more people might be attracted to eat and play turtles, which, in turn, impels a lot of hunters to catch wild turtles. To be worse, once a native turtle is caught, then it is very likely eaten as food or medicine; but if a slider is caught, it is usually released again into waters due to the cheap price. For these reasons, turtle farms and markets should be under strict supervision and control to prevent the farmed sliders from escaping. In addition, an appropriate tax-raise in slider farming has to be advised. Simultaneously, it is also urgent to strengthen the monitoring of farms and markets (including internet markets) and prohibit the sale of endangered wild turtles.

Thirdly, make a scientific management on the activity of freeing animals. Obviously, releasing animals into the wild is an effort which has very strong professional. A variety of factors, such as the origin of animals (alien or indigenous), ecological habits, individual health status, the time and place of releasing, have to be considered by professionals. However, as these aspects involved much knowledge in biology and ecology, non-professionals are almost impossible to get all of them. Thus, we need to take some measures to reduce irresponsible releasing of animals: (1) The releasing activities by religious groups should be supervised by the local government to forbid the releasing of alien species; (2) Establish the Animal Sanctuaries and Rescue Centre, and persons who have seen, bred or captured sliders are encouraged to contact a professional team; and (3) Train law-executors and managers of wildlife to enable them to identify invasive species.

Fourthly, enhance education and awareness of the public. Indeed, most people are not clear on the knowledge and awareness of biological invasions. Thus, public education on invasive species could be an effective strategy for preventing religious release and abandoned pets, particularly invasive species (Liu et al. 2013). In this context, we should pay more attention to the propaganda role of network and media, hold more popular science lectures, and broadcast more scientific documentaries about exotic species to the public. Information campaigns *via* the media should encourage people to better care for their pets/turtles and refrain from releasing them into the wild. All pet shops should be compelled to provide proper care sheets to buyers about how to deal with turtles and other animals, and required to tell customers the potential risk of salmonella infection. Billboards, which prohibit release red-eared slider, should be established in all of the parks and at the edge of natural waters, to inform the people that releasing sliders will cause eco-catastrophe.

21.6 Conclusion

The red-eared slider is an opportunistic omnivorous animal who has a strong competitive ability. This turtle can adapt to various environments, whether it is clear lakes, reservoirs, or muddy ponds, ditches, or even brackish waters in estuary; we could find them in almost all contaminated and uncontaminated waters, which has been become one of the most widely distributed invasive species in the world. On the one hand, the red-eared slider has a extend niche, the various physiological activities in tolerance to different ecological factors, and even in some environmental stresses; on the other hand, the red-eared slider has a strong behavioral adaptability and plasticity, it can adjust and change itself to adapt to all kinds of environmental conditions. The study found that, the red-eared slider can effectively utilize all kinds of resources, usually occupied and ruled the habitats which are the most conducive to its life.

Apart from all the own characteristics of red-eared slider, human factors played a key role in successful invasions. The surveys found that the wild distribution of red-eared slider was mainly concentrated in the waters surrounding the cities with high population and developed economy, and “released by people” is the main way causing sliders into natural environments.

At present, the threats from the red-eared slider are becoming extremely serious. In order to protect our fragile ecosystems and declining biodiversity, we still need to increase investment in scientific research, continue to carry out the studies on demographic characteristics (density, growth rate, survival rate, age composition, sex ratio, diffusion, etc.) of sliders. Monitoring the temporal and spatial changes of population for a long-term, and strengthen efforts to propaganda combining the government and social forces together to control this pest species.

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Chapter 22
Nile Tilapia *Oreochromis niloticus* (L.)

Dangen Gu, Yinchang Hu, Hui Wei, Yunjie Zhu, Xidong Mu, Du Luo, Meng Xu, and Yexin Yang

Abstract Nile tilapia *Oreochromis niloticus* (L.) is one of the most widely cultured and most widespread invasive fish species globally. It was the most common alien fish species in South China where it has successfully established populations in the major rivers, and became the dominant species in many sites. The distribution of Nile tilapia in the wild in China is limited by the temperature and the biodiversity of native fish, so it was abundant in the rivers with lower biodiversity in South China. A series of ecological problems may have caused by the increase of Nile tilapia, such as the decrease of the CPUE (catch-per-unit-per-effort) and the income of fishermen, influence on growth of native fish species, increase of the fishing pressure on native species, and decrease of the water transparency and water quality. In order to control the population of the Nile tilapia in the wild, an organophosphate drug called "Mie fei ling" was developed and used. Meanwhile, science popularization has been used to strengthen the understanding of the public and to avoid Nile tilapia to be released and escaped from aquaculture. In order to reduce the ecological impact, more investigations should be conducted and suitable control methods should also be determined for this invasive species.

Keywords Nile tilapia • Biological invasion • South China • Species diversity • Fisheries

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22.1 Introduction

Tilapia is the common name for nearly 70 species of cichlid fish that are native to fresh waters of tropical Africa, including the mouthbrooding genera *Sarotheredon* and *Oreochromis*, and the substrate-spawning tilapia (Trewavas 1983; Gu et al. 2014a). Tilapia species are popular in aquaculture because they can tolerate a wide range of environmental conditions, have flexible habitat requirements, reproduce shortly after birth, and grow rapidly (Grammer et al. 2012; Gu et al. 2014b). Many tilapia species have been introduced worldwide to increase the supplies of animal proteins (Costa-Pierce 2003; Brinez et al. 2011). For these reasons, tilapia (Nile tilapia, Fig. 22.1) has become one of the most widespread exotic fish, second only to common carp (Rutten et al. 2004). Established tilapia populations have been found in natural waters of many countries, such as the USA, Canada, Australian, China, and Brazil (Costa-Pierce 2003; Canonico et al. 2005; Peterson et al. 2005; Grammer et al. 2012; Gu et al. 2012a).

China has dominated the world's tilapia production and has witnessed a stable annual production of 1–1.5 million metric tons, approximately half of the world total production (Chu et al. 2012; Wang et al. 2014). In China, the tilapia *Oreochromis mossambicus* was first introduced from Vietnam in 1957. Since then, several other tilapia species, such as blue tilapia (*Oreochromis aureus*) and different strains of Nile tilapia (*Oreochromis niloticus*), have been introduced and crossbred (Zhu et al. 2008; Gu et al. 2014a). So far, there have been more than six tilapia species in China, mainly including *O. mossambicus*, *O. aureus*, *O. niloticus*, *Tilapia zillii*, *Sarotherodon melanotheron*, and *Sarotherodon galilaeus* (De Silva et al. 2004; Zhu et al. 2008). These species are cultured in most regions of China, especially in South China (Guangdong, Hainan, Guangxi, Fujian and Yunnan Provinces) where had 81% of the total production in 2013 (Wang et al. 2014). Of these introduced species, Nile tilapia (*O. niloticus*) and its hybrids are most widely distributed and have become dominant fish species in the main tilapia-producing areas such as South China, contributing most to the fish production in China (Zhu et al. 2008; Gu et al. 2014b; Gu et al. 2016).

22.2 Distribution and Invasion of Tilapia in China

22.2.1 Distribution

Wild Nile tilapia is distributed in South China mainly because it cannot survive cold winter temperatures in other regions (Gu et al. 2014b). This tilapia is ubiquitous in the main rivers of South China, such as the Pearl River (with large tributaries that include the Xijiang River, Beijiang River and Dongjiang River), Hanjiang River, Jianjiang River and Nandujiang River (Xu et al. 2006; Gu et al. 2012a, b, 2014b) (Fig. 22.2; Hu et al. 2015). In addition to rivers, many other habitats are also present with this fish, such as ponds, lakes and canals (Fig. 22.3).

Nile tilapia has become the dominant fish species in many rivers, the weight ratio in major rivers of South China was shown in Table 22.1 and Fig. 22.4 (Gu et al.

Fig. 22.1 Nile tilapia

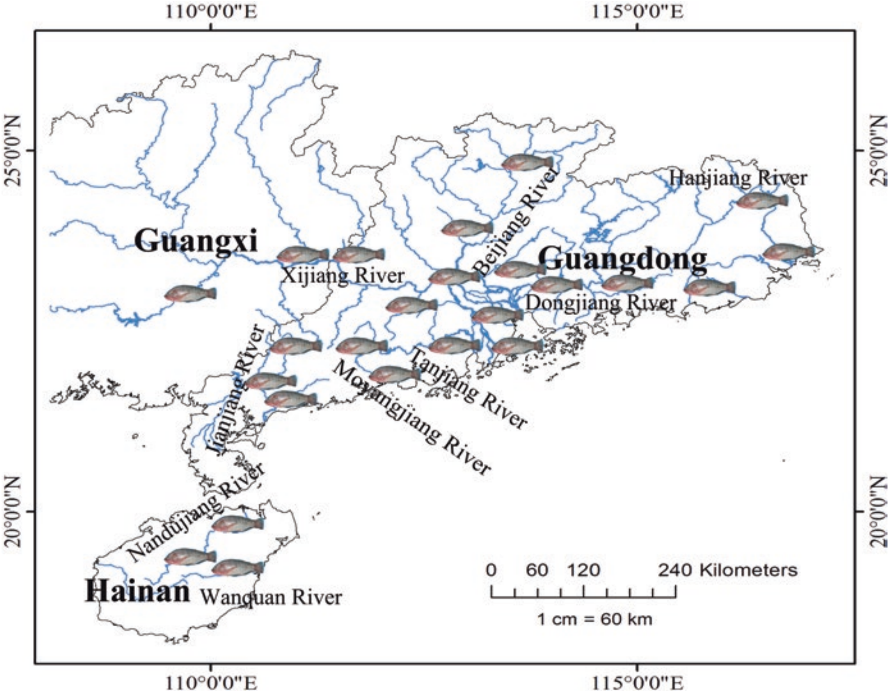

Fig. 22.2 Distribution of tilapia in rivers of South China (According to Hu et al. 2015)

2012a, b, 2014a, b). Nile tilapia was detected as dominant species in many surveyed sites (Fig. 22.2), especially in Meihua and Huazhou (Table 22.1 and Fig. 22.4), which belong to the Jianjiang River basin, one of the most important farming areas of tilapia industry (Chu et al. 2012). As expected, breeding density and the number of tilapia in the Jianjiang River basin were much higher than that in other areas (Chu et al. 2012). This is accordant with previous finding that Nile tilapia was abundant in such rivers that neighbor aquaculture ponds, from which it might escape and reach the rivers through river flooding (Radhakrishnan et al. 2011).

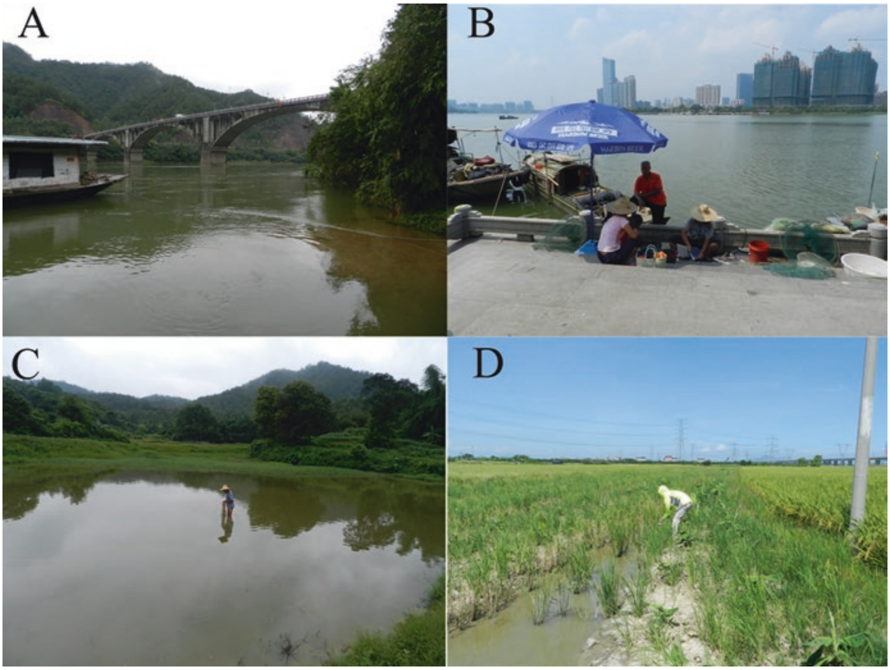

Fig. 22.3 Habitat of wild tilapia (**a**: river, Sanhe, Hanjiang River; **b**: river near city, Huizhou, Dongjiang River; **c**: lake Yangjiang; **d**: canal, Shanwei)

Table 22.1 The weight percentage of tilapia in different sample points (Gu et al. 2012a)

River	Sampling Site	Percentage of Weight (%)
Jianjiang River	Meihua	60
	Huazhou	25
Xijiang Rive	Muzhou	14
	Zhaoqing	<1
Beijiang River	Shaoguan	<1
Dongjiang River	Huizhou	7.8
Tanjiang River	Daze	2
Hanjiang River	Sanhe	22

22.2.2 *Natural Population Established*

Once invasive fish species establish, they are nearly impossible to be eradicated (Zengeya et al. 2013). Nile tilapia populations were found with extremely large size in the major rivers of South China (Liu et al. 2011; Gu et al. 2012a; Tan et al. 2012; Gu et al. 2014b). Moreover, besides mature individuals, many two-year-old

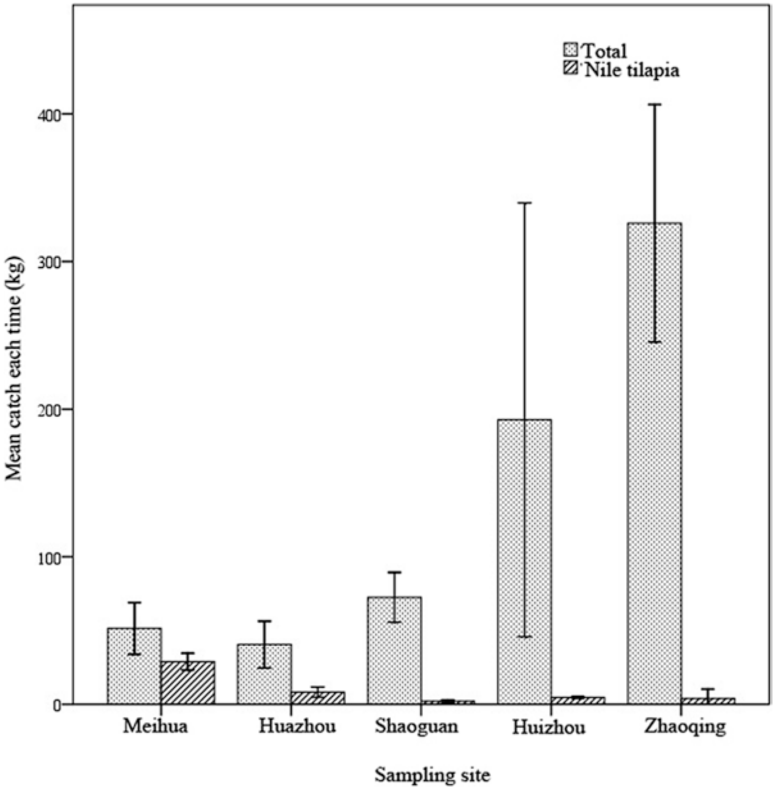

Fig. 22.4 Total catch and tilapia catch (mean ± SD) during each survey at five sites of China (Gu et al. 2014b, Sits were according to Fig. 22.2)

Table 22.2 The age structures of tilapias in different populations (Gu et al. 2012a)

Rivers	Sampling Site	1 year old	2 year old	3 year old	4 year old
Jianjiang River	Meihuazhen	57.89%	42.11%	–	–
	Huazhou	76.47%	14.71%	5.88%	2.94%
Xijiang river	Muzhouzhen	63.64%	9.09%	27.27%	–
	Zhaoqing	–	95%	5%	–
Beijiang River	Shaoguan	93.1%	6.9%	–	–
Dongjiang River	Huizhou	51.92%	44.23%	3.85%	
Tanjiang river	Dazezhen	57.14%	42.86%	–	–
Hanjiang river	Sanhezhen	100%	–	–	–
	Xiyangzhen	75%	25%	–	–

individuals and large numbers of juveniles could also be found in all populations (Table 22.2). Thus, it has been able to overwinter and reproduce in natural waters (Gu et al. 2012a). All results suggest that Nile tilapia has established natural populations in the major rivers of South China (Gu et al. 2012a).

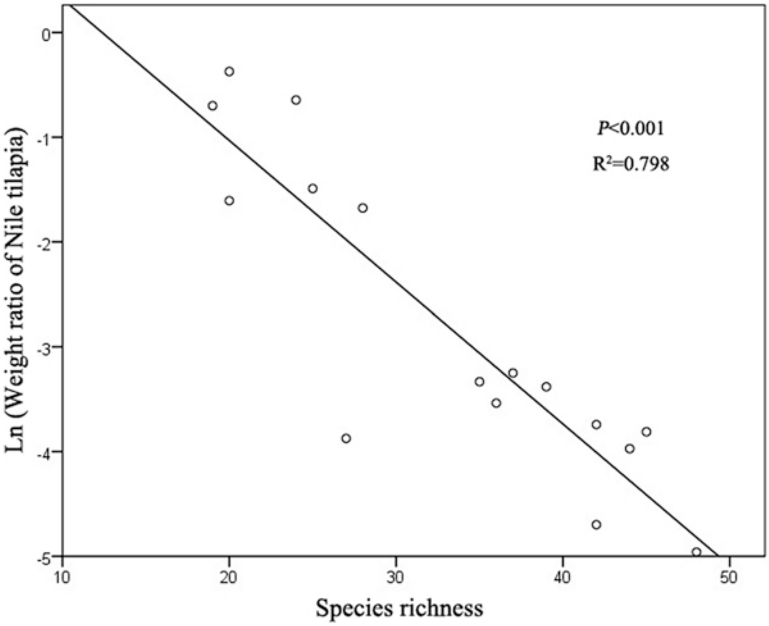

Fig. 22.5 Relationship between species number and the weight ratio of Nile tilapia (Gu et al. 2014b)

22.2.3 *Species Diversity Defends Against the Invasion of Tilapia*

Native communities may prevent invasions through a variety of mechanisms, which are referred as to "biotic resistance" (Elton 1958). Field surveys and laboratory experiments were conducted to test the theory of "biotic resistance" and ascertain the relationship between native species richness and the invasion of Nile tilapia from 2011–2013. In the field, as the richness of native species increased, the biomass of Nile tilapia significantly reduced (Fig. 22.5) (Gu et al. 2014b). Consistent with results from the field, the manipulative laboratory experiment also indicated that the growth rate of Nile tilapia was negatively related to native species richness (Fig. 22.6) (Gu et al. 2014b). All results suggest that species biodiversity represents an important defense against the invasion of Nile tilapia, which is accordant with the theory of "biotic resistance".

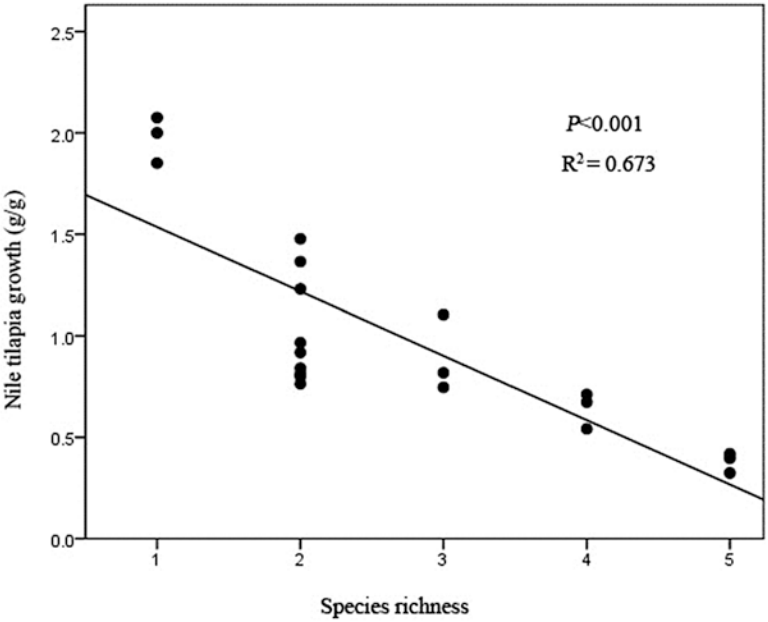

Fig. 22.6 Relative growth of Nile tilapia across different levels of species richness (Gu et al. 2014b)

22.3 Impacts

22.3.1 Impacts on Fisheries

The increase in Nile tilapia in rivers has both affected the biomass of native fish and reduced the income of fishermen (Gu et al. 2015; Fig. 22.7). When the abundance of Nile tilapia increased, the catch-per-unit-per-effort (CPUE) of total species and native species significant decreased (Fig. 22.8) (Gu et al. 2015). The decrease of CPUE leads a loss of the income for fishermen. As wild Nile tilapia is cheaper than native species, the fishermen's income would be reduced when the weight ratio of Nile tilapia increases, even at a same CPUE (Gu et al. 2015). Moreover, the lower price of Nile tilapia will render them to be discarded after being caught, which may result in a vicious cycle that ultimately leads to the collapse of fish stocks (Fig. 22.9) (Gu et al. 2014b, 2015).

Fig. 22.7 Fisheries in river

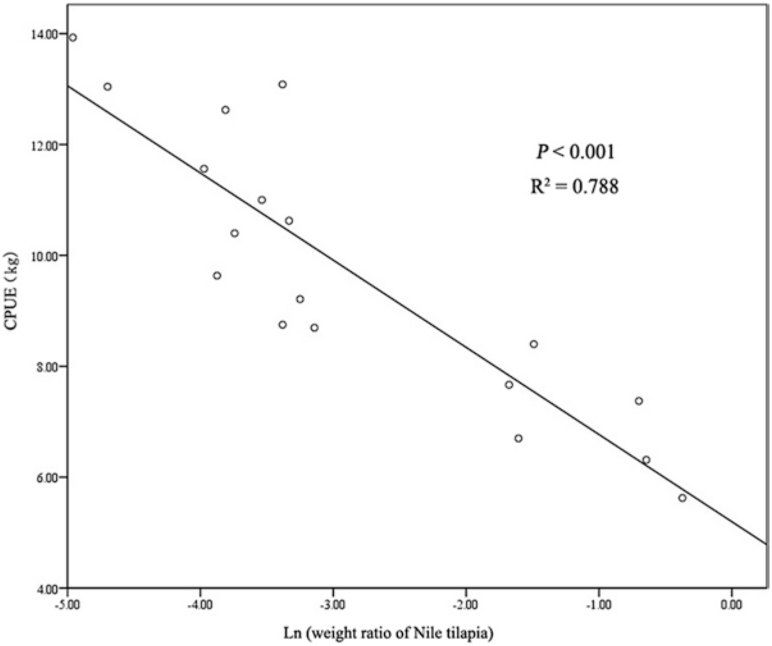

Fig. 22.8 Relationship between CPUE (average catch-per-boat-per-day) and weight ratio of Nile tilapia in 18 filed surveys (Gu et al. 2015)

22.3.2 Impacts on Native Fish Species

The growth and survival of native species also decreased when the Nile tilapia existed (Gu et al. 2015). This is the case for mud carp (*Cirrhina molitorella*), which is the most popular native fish in rivers of South China and has a diet similar with

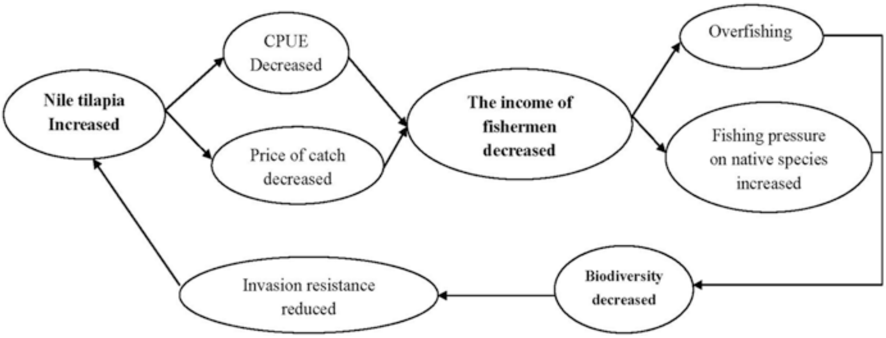

Fig. 22.9 Vicious cycle caused by Nile tilapia (According to Gu et al. 2014b, 2015)

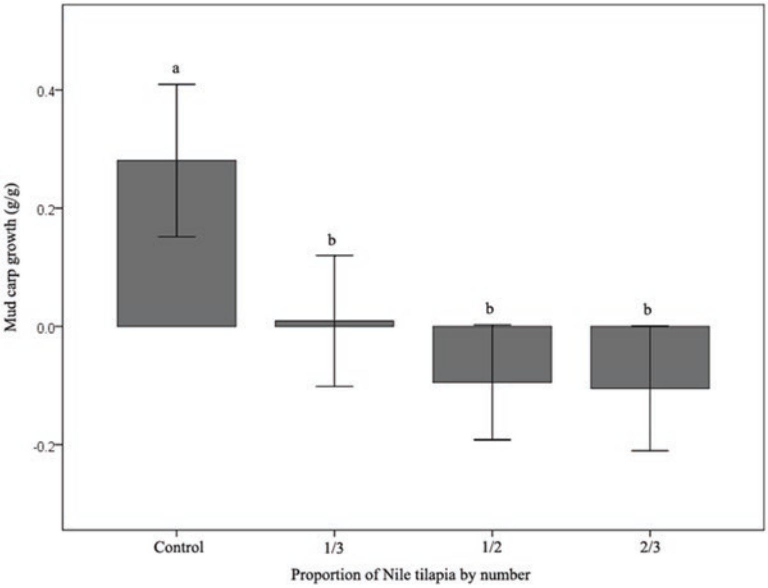

Fig. 22.10 Native mud carp growth in the presence of Nile tilapia (Gu et al. 2015)

Nile tilapia (Chen et al. 1990; Zhu et al. 2008; Li et al. 2009; Liu et al. 2011). The mud carp's growth would be significantly reduced in the presence of Nile tilapia. Such an impact may take place even if food resources are abundant, and it would be worse when food resources are limited (Fig. 22.10) (Gu et al. 2015).

Fig. 22.11 The Jianjiang River which has the largest number of Nile tilapia and the worst water quality in the surveys

22.3.3 *Impacts on Water Quality*

Tilapia can increase total nitrogen (N), total phosphorus (P) and chlorophyll in the reservoirs invaded by such fish (Liu et al. 2008). The possible reason is that excretion can increase nutrient loading, which in turn, enhance phytoplankton growth. As phytoplankton biomass increases, water transparency and water quality decrease (Liu et al. 2008; Yao and Liu 2010). In the previous surveys, it has been found that the biomass of Nile tilapia was associated with the water quality of rivers (Fig. 22.11).

22.4 Control

The serious influence of the invasive Nile tilapia on ecosystems has prompted research on quick and efficient elimination of this species in the wild (Ma et al. 2014). There have been several methods for controlling Nile tilapia, including physical, chemical and biological control, genetic engineering, environmental management and cultural control (Ma et al. 2015a). Yet, most of these methods have not been widely used in China, and the control efficiency is expected to be weak in near future, due to the lack of manpower and money required to implement these methods. Some methods even introduce new harm to ecosystems, such as numerous biological controls adversely affecting non-target native species (Ma et al. 2014, 2015a).

A more effective, target-specific and less persistent organophosphorus pesticide, “Miefeiling”, was screened to control the Nile tilapia in small water bodies. This pesticide has been tested for its acute toxicity on a variety of common fish, such as

Table 22.3 The acute toxicity of "Mie fei ling" on Nile tilapia at intervals of time under the monoculture (Ma et al. 2014)

Drug concentration (mg/L)	Mortality rate (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
0.10	23.33 ± 5.77	36.67 ± 5.77	76.67 ± 5.77	100
0.17	43.33 ± 5.77	56.67 ± 5.77	90.00 ± 10.00	100
0.20	56.67 ± 5.77	83.33 ± 5.77	100	100

grass carp (*Ctenopharyngodon idellus*), carp (*Cyprinus carpio*), crucian (*Carassius auratus*) and loach (*Misgurnus anguillicaudatus*), as well as on two small experimental animals, zebrafish (*Danio rerio*) and White Cloud Mountain minnow (*Tanichthys albonubes*) (Table 22.3). The results showed that Miefeiling had a selective toxic effect on Nile tilapia, possibly by inhibiting AChE activity in this fish. The observed selective toxicity of this pesticide might be related with the sensitivity variance of AChE among different species (Ma et al. 2015a, b). So far, "mie-feilling" has been used in many aquaculture water areas to control the Nile tilapia and has good performances. It has also been recommended to be used for clearing of this fish in the wild.

Careful managing aquaculture ponds should be an important control method, because ponds may serves as an important source for the invasion of Nile tilapia to rivers (Gu et al. 2012b, 2014b). We have to strengthen the awareness of the public so as to reduce Nile tilapia escape from aquaculture facilities. To pursue this goal, lots of science–popularization activities are being organized in China (Hu et al. 2015).

22.5 Conclusions

The introduction of Nile tilapia has made a great contribution to aquaculture and capture fisheries production of China. However, it has become one of the most widespread invasive fish species in this country. Nile tilapia populations have become established in the main rivers of South China and have caused a series of ecological problems. The increase of Nile tilapia had not only affected the biomass of fish species but also reduced the fishers' income. Moreover, expansion of Nile tilapia has also significantly reduced the growth of some native aquatic species, such as mud carp, and thus may threaten the fish biodiversity and fishery resources of this country. Tilapia may also worsen water transparency and water quality.

In order to reduce the ecological impacts caused by the invasion of Nile tilapia, more investigations should be conducted to understand the impact of Nile tilapia on native fish, fisheries and the structure and function of aquatic ecosystems. To reduce the negative effect from this alien species, appropriate measures should be taken to protect the native species biodiversity to prevent its invasions, and breed activities should be more strictly managed to prevent possible escapes. More suitable control methods should also be developed.

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Chapter 23
North African Catfish *Clarias gariepinus* (Burchell)

Dangen Gu, Yinchang Hu, Hui Wei, Yunjie Zhu, Xidong Mu, Du Luo, Meng Xu, and Yexin Yang

Abstract Fast expansion of aquaculture in China largely relies on the introduction and use of non-native species. However, the introduction and use of non-native species may have negative impacts on local ecosystems and societies. *Clarias gariepinus* has been widely farmed in China after introduced in 1980 from Africa. However, during aquaculture, this fish species has escaped from farm facilities to the wild. So far, we have little knowledge on the invasion status of this non-native fish in China. In this Chapter, we review the introduction, spread and impacts of *C. gariepinus* in China, as well as present potential management strategies for this alien fish. Multiple lines of evidence indicate that *C. gariepinus* has been widely distributed and established population in China and the negative impacts of this alien fish in local ecosystems have been detected. Therefore, we suggest that suitable risk assessment and early waning programs should be well developed to prevent the spread and species-specific fishing tools could be designed to control this alien fish in the wild. We also call for deep investigations on mechanisms of invasions and the technologies of management for *C. gariepinus*, as well as government and aquaculture industry for proper risk assessment and sound management for sustainable development of aquaculture.

Keywords *Clarias gariepinus* • Aquaculture • Non-native fish • Management

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23.1 Introduction

Species introduction has brought billions of profits that support economies throughout the world (Ding et al. 2008). However, species invasions have become an unintentional consequence of commerce that human beings have to face, and they will become worse as global trade and transportation are continuously intensified. The costs of invasions have frequently outweighed the economic benefits derived from species introductions (Perrings 2010, Kang et al. 2015). Thus, while attempting to promote economic activities, policy-makers have to consider the threats that are possibly imposed by species invasions. Fish is one group of aquatic animals most extensively introduced in the world, which provide almost half of the aquatic food consumed worldwide (FAO 2012). Fish introductions will continue worldwide to meet demands for fish products, which may increase ecological risks and biodiversity loss in the introduced ecosystems (Gozlan et al. 2010). In China, aquaculture has expanded fast in the past decades both in production and farming area. In 2011, this industry produced more than 50 million tons with a value of US$ 64 million in the country, nine and 15 times higher respectively than those 25 years before (China Fisheries Yearbook 2012). However, around a quarter of the fishery production in China is derived from farming of nonnative species (Lin et al. 2015).

Clarias gariepinus, or African sharptooth catfish (Clariidae), was first introduced into Guandong province of China in 1981. It has escaped from farming ponds and established in the wild in South China (Radhakrishnan et al. 2011). Yet, little specific regulations have been suggested about the prevention or eradication of this fish species from natural ecosystems. In this chapter, we review the introduction, spread and impacts of *C. gariepinus* in China. We also discuss management strategies with the aim of increasing the public's awareness of this non-native species and giving policy-makers suggestions for the management of this fish.

23.2 Distribution and Invasions of *Clarias gariepinus* in China

C. gariepinus is native to Africa, which was introduced into Europe, Asia and Latin America for aquaculture (Verreth et al. 1993; Vitule et al. 2006) (Fig. 23.1). So far, the distribution range of *C. gariepinus* in Africa has extended, and it has occurred naturally in South Africa (Huchzermeyer 2012; Wartenberg et al. 2013), Asia (Radhakrishnan et al. 2011; Ng et al. 2014) and South America (Vitule et al. 2006).

In China, *C. gariepinus* is a popular economic fish mainly owing to its sound biological characteristics for aquaculture, such as large body size, rapid growth, good meat quality, and tolerance to low water-dissolved oxygen and low temperature (Li et al. 1984). So far, *C. gariepinus* has been widely farmed in more than 19 provinces. However, it has escaped from farming ponds and entered natural water bodies through river flooding (Vitule et al. 2006). According to the fish surveys across the country, *C. gariepinus* has occurred in South China (Fig. 23.2), such as Pearl River Basin including Xijiang River (Zhaoqing and Muzhou Reach), Dongjiang River

Fig. 23.1 *Clarias gariepinus*

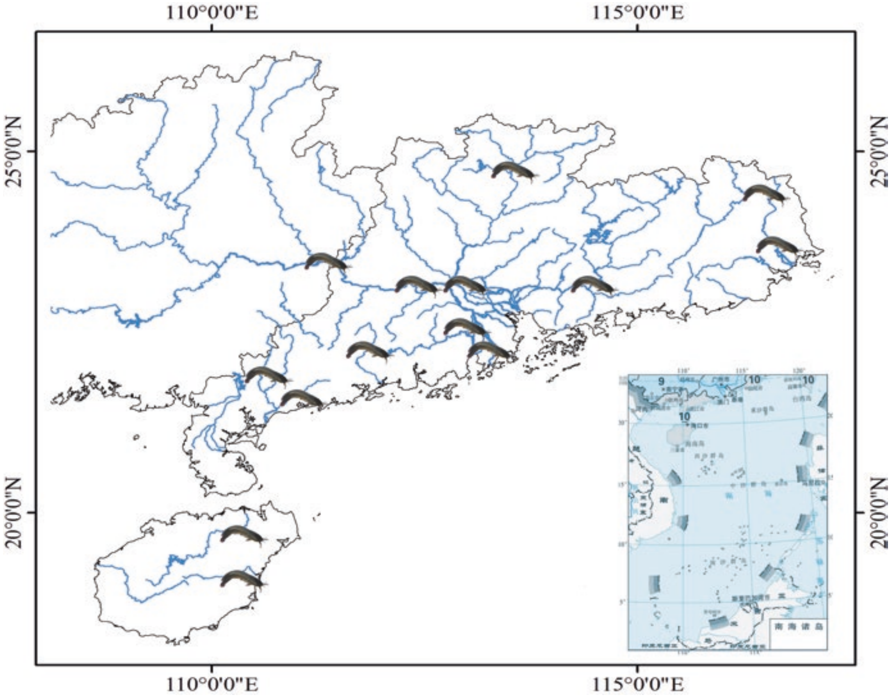

Fig. 23.2 Distribution of *C. gariepinus* in the rivers of South China (According to Hu et al. 2015)

(Huizhou Reach), Beijiang River (Shaoguang Reach), Jiangjiang River (Meihuajiang and Huazhou Reach), Tanjiang River (Dazhe Reach) (Gu et al. 2012) and Xijiang River (Yongjiang, Yujiang, Liujiang and Hongshuihe Reach) (Wang et al. 2007). It has also been observed in the upper Yangtze River (Yibin and Zhigui Reach), lower Yangtze River (Anqing Reach) (Yang 2012), Yellow River (from Xiaolangdi to estuary Reach) and Weihe (Henan Reach) (Lv et al. 2012). Radhakrishnan et al. (2011) and Zhu et al. (2016) suggests that *C. gariepinus* may potentially reproduce and even have established wild populations in south China, because they found mature females and individuals with older than two-yearin these areas.

The rapid spread of *C. gariepinus* in the introduced range may be mainly attributed to its biological characteristics. First, *C. gariepinus* can live in various types of aquatic habitats. Adults inhabit mainly in quiet waters such as lakes and pools and prefer rather shallow and swampy areas with a soft muddy substrate, yet *C. gariepinus* can also live in fast flowing rivers and rapids. Second, *C. gariepinus* can tolerate extreme water conditions, such as hypoxia and low temperature. It has accessory breathing organ which enables it to breath under low water-dissolved oxygen (Donnelly 1973). Xia (2012) found that, as compared with other *Clarias* spp. such as *C. fuscus*, *C.gariepinus* has higher iron concentrations (*C gariepinus* > Mule fish F1 > *C. fuscus*) and iron saturation degrees (*C. gariepinus* > *C. fuscus* > Mule fish F1), which would assist the fish to tolerate hypoxia and thus allow it to survive harsh conditions, such as polluted water bodies. *C. gariepinus* can tolerate low temperature at 6.5–12 °C (Na-Nakorn et al. 1998). Third, *C.gariepinus* has several life history features that are benefit for population development, including cross-habitats foraging and high growth rate. As an omnivorous fish, *C.gariepinus* preys insects, plankton, invertebrates, small-size fish and rotting flesh (Radhakrishnan et al. 2011; Kadye and Booth 2012). *C. gariepinus* has a growth rate five to sixfolds higher than native catfish (Ma 1998). Additionally, this fish species has a high level of fecundity which can spawn 50,000–100,000 eggs each time (for a female with one kilogram in weight), and adults can spawn three to four times each year (Ma 1998). All the biological characteristics mentioned above make *C. gariepinus* become invasive after escaping from aquaculture facilities to natural water bodies and allow it to survive in the wild.

23.3 Impacts

Clarias gariepinus has negative impacts on native species diversity through predation. *C.gariepinus* has large body size, ferocious habit and big appetite, thus it can kill and/or prey on a great number of native species, including small fish, aquatic invertebrates, zooplankton, algae, phytoplankton and macrophytes (Radhakrishnan et al. 2011; Kadye and Booth 2012). Additionally, *C. gariepinus* may compete with indigenous fish species for food and space (Radhakrishnan et al. 2011). For instance, *C. gariepinus* had higher feeding efficiency than native catfish species such as *C. fuscus* and *Peltobagrus fluvidraco*, which share the habitats with the African catfish in the Pearl River basin (Radhakrishnan et al. unpublished). In Singapore, a severe decline was observed in the distribution of a native fish species *C. batrachus* as the distribution of *C. gariepinus* increased sharply, which was thought to be partially resulted from interspecific competition (Ng et al. 2014).

The available evidence clearly illustrates that *C. gariepinus* has caused negative impacts on native ecosystems by invading and occupying habitats that are shared by *C. gariepinus* and native fish, as well as by killing and preying on endemic species. However, the public has not realized the possible negative impacts caused by this alien fish and the potential risk to local aquatic ecosystems. Therefore, further studies are needed to investigate the mechanism of *C. gariepinus* establishment and spread in local aquatic ecosystems, as well as the potential impacts on natural ecosystems and economy.

23.4 Control and Management

Although the negative impacts of alien invasive species have been well known, alien fish are often introduced into new water bodies for aquaculture without considering the potential influence on local ecosystems (Xu et al. 2006). *C. gariepinus* is a representative example: farmed individuals escape from aquaculture facilities to natural water bodies through flooding, poor aquaculture management, and intentional release. Yet, there have been several methods for the control and management of this alien fish in China, such as improvement of aquaculture management and public promulgations for preventing alien fish. Generally, the strategies of controlling and managing *C. gariepinus* mainly include prior procedures and eradication or extirpation technologies, such as mechanical removal, physical control, chemical control and biological control.

Firstly, risk assessments and early detection programs are the prior procedures in fighting against invasive species. Risk assessment has been widely used for preventing the potential noxious alien species, such as Weed Risk Analysis (Pheloung et al. 1999) and Fish Invasion Screen Kit (FISK) (Copp et al. 2005, which based on the biogeography and history of the species to distinguish the potential invasive species (Pheloung et al. 1999, Copp et al. 2005). FISK is a specific decision-making support tool to screen alien fish which have potential invasiveness. This tool has been successful applied in tropical zone (Troca and Vieira 2012), sub-tropic zone (Onikura et al. 2011), and temperate zone (Mastitsky et al. 2010). Early detection is determined by the probability that the surveillance system will detect at least one individual of the target species, given it is present in the sampling frame when the population is at a specific threshold (Jarrad et al. 2011). Environmental DNA (eDNA) is a novel and powerful tool for early detection of non-native species which assay for the presence of species-specific DNA fragments within a sample of bulk environmental material (i.e., water or suspended solids) (Ficetola et al. 2008). This technology has been successfully applied to detect alien species (Chown et al. 2008; Turner et al. 2015). For example, Turner et al. (2015) has successful applied this approach to detect bigheaded Asian carp (*Hypophthalmichthys* spp.) in sediments up to 132 days after carp removal in experimental ponds and natural rivers. However, there is little report about the potential adverse impacts of *C. gariepinus* before introduction, as well as its potential distribution range after the population establishment. Thus further studies are needed for predicting the risk and detecting of *C. gariepinus* invasions.

Secondly, eradication is an approach for complete removal of all individuals of a distinct population. This measure is often used for the control of invasive species that have established population or caused harms in local ecosystems (Simberloff et al. 2013). For alien aquatic species, the eradication technologies tools included barriers (physical barriers, electrical barriers, and interstate/interisland biosecurity barriers) to preventing spread, chemical control (using rotenone, antimycin, saponins, 3-trifluoromethyl-4-nitrophenol), biological control (using predatory fish, pheromone traps, species-specific pathogens, regulating habitats, adopting immunocontraceptives and genetic modification) and physical removal (complete dewatering, explosions netting, angling and electrofishing) (Clout and Williams 2009, Gherardi et al. 2011). There are several successful examples based on these measures. For instance, a sterile-male-release technique was used to control the introduced sea lamprey (*Petromyzon marinus*). By injecting with the chemosterilant bisazir (P, P-bis (1-aziridinyl)-N-methylphosphinothioic amide from 1991 through 1999 in the Great Lakes, *P. marinus*'s reproduction was reduced and fewer recruits were produced (Twohey et al. 2003). Large barriers (e.g., waterfalls) could significantly reduce the movement of invasive crayfish *Procambarus clarkii* between pools (Kerby et al. 2005). Liang et al. (2013) implemented a rice-duck mutualism in paddy fields to control the invasive snail *Pomacea canaliculata*; ducks preyed *P. canaliculata* and destroyed the habitat required for its oviposition, thereby reducing the population density of this snail. For *C. gariepinus*, we suggest designing barriers to prevent its spread. In addition, in order to remove it from the wild, species-specific fishing tools might be an ideal approach, because this fish can serve as food for local people. But so far few measures have been taken to eradicate *C. gariepinus* in natural ecosystems in China, despite that this species has caused negative impacts. Therefore, more efforts are needed to search suitable methods to prevent and control this alien fish.

23.5 Conclusions

Aquaculture is one of the most important parts of global food economy, especially in China where aquaculture production holds a great proportion of the world production (Lin et al. 2015). The fast development of aquaculture largely relies on the introduction and use of non-native species, which may have potential negative impacts on local ecosystem and society (Shelton and Rothbard 2006). Thus there is a paradox that introduced non-native species play a significant role on the fast expansion of aquaculture, while negative effects accompanying with unregulated introduction and irresponsible use of non-native species are increasing in number and affected area (Lin et al. 2015). However, owing to the lag phrase during the processes of biological invasions, it is difficult to detect the harm caused by these non-native species, until leading to a disaster (Simberloff 2011). Therefore, the potential adverse impacts of introduced aquatic animal on local ecosystems and economy are usually overlooked. Multiple lines of evidence indicates that *C. gariepinus* has been widely distributed and established populations in China and the negative impacts of this alien fish in local ecosystems have been detected. Therefore, it is an urgent to strengthen research on the mechanism of invasions and the technologies of management for *C. gariepinus*. Moreover, government and aquaculture industry should implement proper risk assessment and sound management for sustainable development of aquaculture.

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Chapter 24
Common Ragweed *Ambrosia artemisiifolia* L.

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Abstract *Ambrosia artemisiifolia* L., an annual herb native to North America, has caused serious threat to native ecosystems, human health, agriculture and livestock breeding in China. In this chapter, we review the damages and invasion mechanisms of this invasive weed, as well as research progress in biological control and integrated management in China. Finally, we propose research perspectives on *A. artemisiifolia* management in the future.

Keywords *Ambrosia artemisiifolia* • Biological control • Replacement control • Biological control agent • Replacement plant

24.1 Introduction

The common ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), is an annual herb native to southern parts of North America (Bassett and Crompton 1975). It is one of the most noxious weed in agriculture around the world (Chollet et al. 1999; Brückner et al. 2003; Török et al. 2003; Wan et al. 2005). In China, this weed was unintentionally introduced into southeastern coastal region in the 1930s (Wan et al. 1993), and has raised great awareness as an invasive plants since 1980s. Now, it has spread to 21 provinces in middle and eastern part of the country (Zhou et al. 2009, 2011a) (Fig. 24.1). It is a quarantine agricultural pest in China because of its significantly negative effects on agricultural loss, a notorious allergen to human health and impacts on biodiversity (Wan et al. 1995; Ma et al. 2008b; Ministry of Agriculture of China 2006).

Artificial uprooting measure was conducted from late 1980s to early 1990s in order to suppress *A. artemisiifolia* populations in China. Thereafter, effective herbicides were used. However, despite the availability of chemical and mechanical methods, sustainable control strategies were not available before 2007 (Zhou et al. 2009). In the past decade, biological control of this weed has received more attention in China, and several classical biological control programs have been implemented

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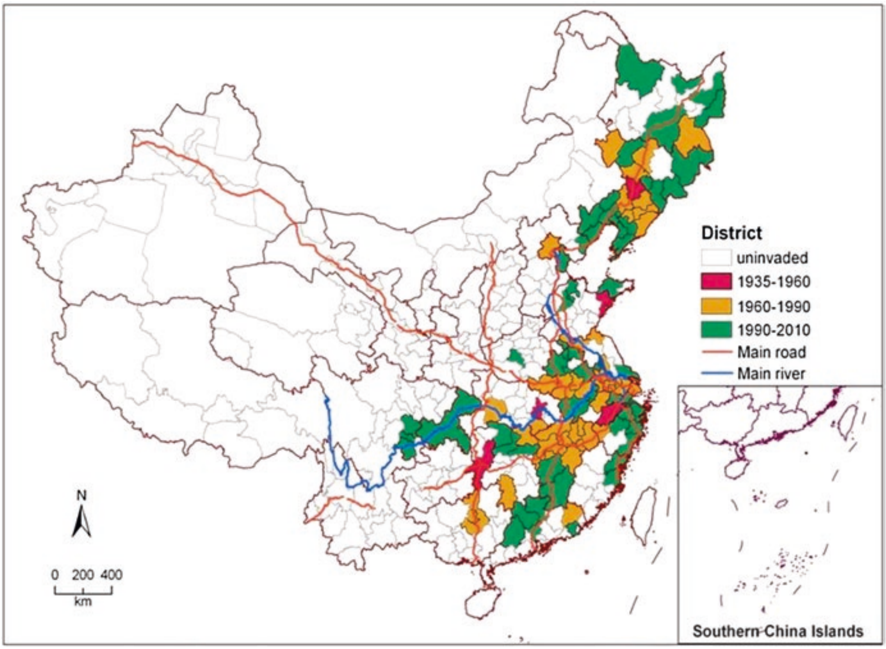

Fig. 24.1 Distribution of *Ambrosia artemisiifolia* L. (Zhou et al. 2015a, b)

(Zhou et al. 2009). Due to the high efficiency, the use of natural enemies has been the sustainable management strategy of *A. artemisiifolia* in China. In this chapter, we briefly review the damages and invasion mechanisms of this invasive weed in China, as well as the research progress in biological control and IPM.

24.2 Damage of *A. artemisiifolia*

Ambrosia artemisiifolia seeds can mix with crop seeds and then invade crop fields (e.g. corn and soybean fields). It can also invade vegetable fields, orchards, mulberry fields, nursery and pasture, *etc*. (Fig. 24.2a). It strongly inhibits cultivated crops and wild plants because it has a strong root system and a large above-ground vegetative biomass (Ma et al. 2008a). Crop yield may be seriously reduced owing to intensive competition with *A. artemisiifolia*. For example, it was reported that the yield of corn (Wan et al. 2005), snap bean (*Phaseolus vulgaris* L.) (Evanylo and Zehnder 1989) and soybean (Wan et al. 2005) was significantly dropped when *A. artemisiifolia* occurred during the growth stage of these crops.

Since *A. artemisiifolia* grows rapidly at late stages, it can remarkably suppress native annual plants and become dominant plant species. Diversity of native plants in invaded fields would decline significantly (Fig. 24.2b), further resulting in a sim-

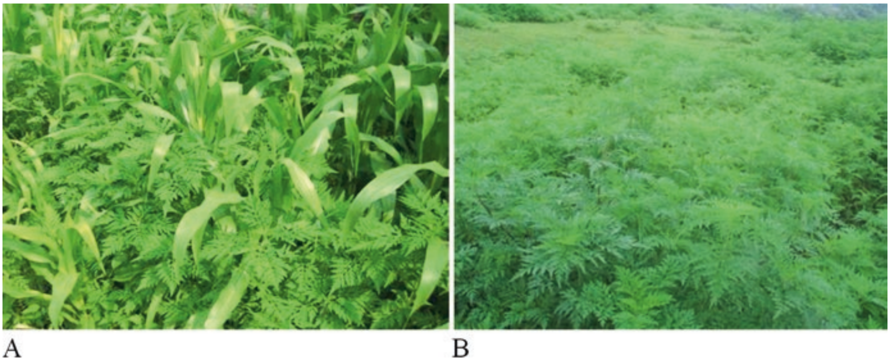

Fig. 24.2 *Ambrosia artemisiifolia* invades the corn field (**a**) and destroys diversity of native plants (**b**)

ple community structure. In addition, diversity of soil animals also significantly decreased in the areas invaded by *A. artemisiifolia* in China (Sun et al. 2002, 2006). On the other hand, *A. artemisiifolia* pollen is one of the most problematic aeroallergens throughout its range (Wan et al. 1993, 2005; Karnkowski 1999; Zhou et al. 2015a, b), which can induce potential severe health problems (Bohren et al. 2006). In China, 2–3% of the population, ca. 14.5 million persons, in the *A. artemisiifolia* occurring regions may suffer from allergy, costing nearly 1.45 billion US dollars annually for prescription drugs in China (Zhou et al. 2011a).

24.3 Invasion Mechanisms

24.3.1 Spread of **A. artemisiifolia** *Seeds*

A. artemisiifolia reproduces by seeds, and seeds can go into secondary dormancy and are capable of maintaining vitality in the soil for 5–30 years (Karnkowski 1999). Seeds can be dispersed by birds, water currents and strong winds. They can be also dispersed through exchanges of contaminated crop seeds, forage and fodder (Karnkowski 1999; Zhou et al. 2015a, b). *A. artemisiifolia* has a relatively high reproductivity, with one single plant capable of producing nearly 100,000 seeds.

24.3.2 Soil Ecological Environment Facilitate the Invasion and Expansion of **A. artemisiifolia**

The invasion and expansion of *A. artemisiifolia* may depend on soil ecological conditions (soil microorganisms, nutrient availability, *etc.*). It is well known that arbuscular mycorrhizal fungi (AMF) may play important roles in invasion processes of some plants. As *A. artemisiifolia* is known to be colonized its population by AMF within its native range, AMF was perceived to facilitate the invasion of this plant. Greenhouse experiment showed that AMF could positively affect growth and development of *A. artemisiifolia* (Fumanal et al. 2006). In addition, the symbiosis between *A. artemisiifolia* and AMF may increase the soil nitrogen acclimation, which enhances resource capture of the plant by increasing specific leaf area, and this effect would be more significant at low soil nitrogen content. Therefore, the role of AMF for *A. artemisiifolia* growth was considered to be more evident in low-nitrogen environments (Huang et al. 2010).

In addition, the invasion of *A. artemisiifolia* can alter the soil microbial community at a certain degree that favors itself while inhibiting native plant species (Li et al. 2014). The change in the microbial community improved the activities of urase, phosphatase and invertase in the soil (Li et al. 2009). These enzymes can accelerate the accumulation of available N, P and K, thus concentrations of available N, P and K in the soil were significantly higher at the sites heavily invaded by *A. artemisiifolia* than at those with only native plants (Li et al. 2014). In addition, *A. artemisiifolia* plants secrete phytotoxic (allelopathic) substances that can inhibit the growth of native plants (Karnkowski 1999).

24.3.3 Benefit from the Escape of Natural Enemies

The enemy release hypothesis support that invading species would benefit compared to their native counterparts if they lose their herbivores and pathogens during the invasion process (Agrawal et al. 2005). A total of 450 insect, mite and fungal species associated with *A. artemisiifolia* have been found since 1965, which had greatly suppressed the expansion of *A. artemisiifolia* in North and South America (Goeden and Andres 1999). In China, however, escape from natural enemies probably has facilitated the population establishment of this plant (Ma et al. 2008a, b; Gerber et al. 2011). This was considered as one of the most important reasons for the successful expansion of *A. artemisiifolia* in this country.

24.4 Biological Control

To control *A. artemisiifolia*, five beneficial insect herbivores were introduced into China from 1987 to 1989, i.e., *Zygogramma suturalis* (Fabricius) (Coleoptera: Chrysomelidae), *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae), *Liothips* sp. (Thysanoptera: Phlaeothripinae), *Euaresta bella* (Loew) (Diptera: Tephritidae) and *Tarachidia candefacta* Hübner (Lepidoptera: Noctuidae) (Wan et al. 1993, 2005; Ma et al. 2008a). *Ophraella communa* LeSage (Coleoptera: Chrysomelidae), native to North America (LeSage 1986; Futuyma 1990), is also a biological control agent of *A. artemisiifolia* (Goeden and Ricker 1985; LeSage 1986; Zhou et al. 2010). It was first detected in 2001 in the suburb of Nanjing city, Jiangsu province (Meng and Li 2005). It feeds on *A. artemisiifolia* leaves (Takizawa et al. 1999; Yamazaki et al.

2000; Dernovici et al. 2006; Zhou et al. 2011b), killing plants when its adults and larvae reach a high density (Lesage 1986; Teshler et al. 2000, 2002; Zhou et al. 2009).

Lots of work has been conducted for the use of these biological control agents, biological control agents have been employed in practice (Reznik 1991; Igrc et al. 1995; Goeden and Andres 1999; Wan et al. 1995; Ma et al. 2008b; Teshler et al. 1996, 2000; Moriya 1999; Zhou et al. 2011a). To better perform biological control of *A. artemisiifolia*, we introduced host specificity and mass rearing and control efficacy of these biological control agents in this section.

24.4.1 Specificity of Biological Control Agents

Host specificity is a prerequisite condition that an insect herbivore can become a biological control agent of the weed. To confirm the safety of the introduced several insect herbivores of *A. artemisiifolia*, their host specificity was successively tested in the laboratory and in the field. Host specificity of *Z. suturalis* was tested on 74 plant species/varieties prior to field releases, where the beetle was found to feed only *A. artemisiifolia*. It did not accepted *A. trifida*, another invasive plant in China, as a host (Wan et al. 1989). In choice-tests, *E. strenuana* could attack sunflowers and produced adults on the plants in the greenhouse (Wan et al. 1995), but its larvae did not develop in a field cage test (Wan and Wang 2000). No eggs were laid on sunflowers in the open field experiment (Wan and Wang 2000). According to host specificity and risk analysis of *E. strenuana* on 36 plant species, this moth is an safe biological control agent of *A. artemisiifolia* because its larvae fed only *A. artemisiifolia* and *Xanthium sibiricum* (Ma et al. 2002; Zhou et al. 2009). Of the other three introduced biological control agents, *Liothips* sp. and *T. candefacta* had low host specificity, but *E. bella* is difficult to be raised (Wan et al. 1993).

Hu and Meng (2007) tested host specificity of *O. communa* on 52 plant species in the laboratory. They found the most preferable host is *A. artemisiifolia*, followed by *X. sibiricum*, and it did not pose risk to other plant species. However, another study in Australia showed that *O. communa* could attack and complete life cycle on sunflower. Consequently, the beetle was subsequently rejected as biological control agent (Palmer and Goeden 1991). However, more recent studies indicated that the risk of *O. communa* adult attacking sunflowers was negligible, and it is a potential biological control agent of *A. artemisiifolia* (Dernovici et al. 2006; Zhou et al. 2011b).

24.4.2 Mass Rearing of Biological Control Agents

Biological control of *A. artemisiifolia* cannot achieve without mass rearing of the biological control agents. *O. communa*, *E. strenuana* and *Z. suturalis* could be mass reared in greenhouse, using *A. artemisiifolia* plants at a height 70–80 cm (Wan et al. 1995; Ma et al. 2008b; Zhou et al. 2009). Both *E. Z. suturalis* and *O. communa* adults lay eggs on leaves and complete their life cycle on leaves within 25–30 days. As adults emerge, they are collected, placed in transparent plastic boxes containing fresh *A. artemisiifolia* leaves, and then transported and released into areas invaded by *A. artemisiifolia*.

E. strenuana adults lay eggs on leaf buds and complete its life cycle in the stem within 40 days. *A. artemisiifolia* branches harboring old larvae are cut off to a length 30–40 cm, placed into paper boxes and then transported (Zhou et al. 2009).

24.4.3 Control Efficacy of Biological Control Agents

Since *A. artemisiifolia* is an annual weed and reproduces by seeds, an effective biological control needs to prevent *A. artemisiifolia* plants producing seeds. At present, *O. communa* has the best biological control agent of *A. artemisiifolia*, and it performs a good control efficacy on *A. artemisiifolia*. Previous study demonstrated that prior to seed production, 100% of *A. artemisiifolia* could be killed by *O. communa* within 47 days when releasing 12 beetles per plant, or within 85 days with an average of 1.07 beetles per plant (Guo et al. 2011; Zhou et al. 2014).

E. strenuana is also an important biological control agent of *A. artemisiifolia*. Previous cage experiment showed that the mortality of plants were 78.0, 95.7 and 99.5% after 12 days in the treatment plots infested with 20, 40 and 60 larvae when *A. artemisiifolia* was weak at the seedling stage (Wan et al. 1993). After *E. strenuana* were released in Hunan and Liaoning Provinces in 1993 (Ma et al. 2008a, b), sustainable populations have been established in the field. Unfortunately, it had a poor suppressive ability on *A. artemisiifolia*, thus it cannot sufficiently control *A. artemisiifolia* in China (Wan et al. 2005; Zhou et al. 2014).

Owing to different spatial niches of *O. communa* and *E. strenuana*, they can coexist in the same *A. artemisiifolia* plant. Consequently, it has been recommended to joint releasing these two agents to get a high control efficiency. A field plot experiment showed that releasing an average of 0.53 *O. communa* with 0.53 *E. strenuana* per plant and 12 *O. communa* with 16 *E. strenuana* per plant at early (60–70 cm tall) and later (90–100 cm tall) growth stages could kill all plants prior to seed production. Thus, joint releasing of the agents at a suitable density can kill all *A. artemisiifolia* plants prior to reproduction (Zhou et al. 2014). In recent years, the joint biological control technology of *A. artemisiifolia* with *E. strenuana* and *O. communa* has been applied in many provinces of southern China. For example, in Laibin, Guangxi Zhuang Autonomous Region, all *A. artemisiifolia* plants in the biological control plots were killed by the end of August when the two biological control agents were released on 24 May in 2009 at approximately 1100 moths and 1100 beetles per ha (Zhou et al. 2011c). The technology has also been applied at Dajing, Xinshi and Zhifeng in Miluo City of Hunan province, where all plants were killed prior to flowering or fructicative period (Zhou et al. 2011d). Moreover, as *O. communa* and *E. strenuana* could overwinter successfully, they can suppress significantly *A. artemisiifolia* populations in the next year in southern China (Zhou et al. 2011a).

Another biological control agent, *Z. suturalis* has a good effect on *A. artemisiifolia* in the cage experiment. Subsequently, a total of 30,000 *Z. suturalis* adults were released in Shenyang, Tieling, Dandong and Nanjing, Changsha, Linxiang and Beijing in 1989–1991. Unfortunately, both larvae and adults of the beetle were not active, and thus vulnerable to the attack of spiders, lacewings and assassin bugs in the field. Therefore, it has not established sustainable populations at the release sites (Wan et al. 1995; Ma et al. 2008b).

24.5 Replacement Control

At present, several native plants with economic or ecological values have been chosen for replacement control of *A. artemisiifolia* in China, including *Amorpha frutiocosa*, *Helianthus tuberosus* L., *Coronilla varia*, *Poa pratensis* L., *Paspalum natatu* and *Pennisetum americanum* x *P. purpureum* (Ma et al. 2008a; Zhou et al. 2011a). *A. frutiocosa* and *H. tuberosus* have been used in the highway from Shenyang to Dalian in Liaoning province. *P. americanum* x *P. purpureum* can be used for rebuilding the areas invaded by *A. artemisiifolia* in flood lands, wastelands, *etc*. (Zhou et al. 2011a).

24.6 Integrated Management

Since *A. artemisiifolia* can grow in different habitats, control strategies should change according to various habitats. In farmlands, orchards and scenic spots, biological control should be the first option. As to the control of *A. artemisiifolia* in the south, central and north subtropics of China, we recommend releasing 1200 *O. communa* + 1200 *E. strenuana* per hm^2, 3000 *O. communa* + 3000 *E. strenuana* per hm^2 and 4500 *O. communa* + 4500 *E. strenuana* per hm^2, respectively, into the areas invaded by *A. artemisiifolia* in the early spring. The populations of the two biological control agents increased with changing seasons, their populations reached the peak, and most of *A. artemisiifolia* plants were killed in summer. At this time, the two biological control agents were collected in the biological control areas, and then released into the other areas invaded by *A. artemisiifolia* (Zhou et al. 2011a).

On roadsides, *A. frutiocosa* or *C. varia* can be planted in the diffusion frontiers of *A. artemisiifolia*. This would hopefully replace *A. artemisiifolia* and thus prevent its spread. In the embankments and wastelands invaded by *A. artemisiifolia*, *P. americanum* x *P. purpureum* can be planted for replacement control of *A. artemisiifolia*. In addition, biological control can be employed in all of these areas. Similarly, in scenic spots, both biological control and replacement control can be used (Zhou et al. 2011a).

24.7 Conclusions and Perspectives

In China, spread of *A. artemisiifolia* has been greatly suppressed by reducing flowering, seed set and dispersal (both naturally and by human activities). In order to mitigate crop losses and threats to human health, ragweed biomass needs to be reduced quickly. Now, biological control of *A. artemisiifolia* has been applied in the southern China, where biological control agents (*O. communa* and *E. strenuana*) have successfully suppressed *A. artemisiifolia.* Since *A. artemisiifolia* is still abundant in the northern China due to lack of biological control agents, its population is quickly spreading in these areas. The use of herbicides or replacement plants is effective in highly infested non-crop areas such as grassland, wasteland, roadsides and riverbanks, in northern China to reduce flowering, pollen production and seed set.

In recent years, we found that cold hardiness of *O. communa* can be promoted by cold acclimation in previous generation, and it might counter-balance reduced survival in the next generation, especially when insects are tracking their host-plants into colder climates (Zhou et al. 2013). Thus we hope that a cold-resistance population of *O. communa* will be achieved by cold acclimation in order to control *A. artemisiifolia* in the northern China. Management of *A. artemisiifolia* largely relies on the integration of biological control, replacement control and chemical control in the future.

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Chapter 25
Crofton Weed *Ageratina adenophora* (Sprengel)

Guoqing Yang, Furong Gui, Wanxue Liu, and Fanghao Wan

Abstract The crofton weed, *Ageratina adenophora* (Sprengel), a plant native to central Mexico and Costa Rica, has invaded more than 30 countries and regions of tropical and subtropical zones. In the 1940s, it was introduced from Myanmar into the south Lincang (e.g. Cangyuan and Gengma) of Yunnan Province, China. Subsequently, this weed has widely spread throughout Southwestern China including Yunnan, Guizhou, Sichuan, Guangxi, Xizang Provinces and Chongqing, with an ongoing spread eastward and northward at a speed of 20 km per year. The crofton weed has caused serious economic losses to agriculture, forestry and livestock, and severely damaged the ecology and environment of China's native habitats, e.g. exposure to or consumption of this weed makes livestock sick. In addition, the crofton weed can establish monocultures in places where diverse native communities once flourished. Potential invasive mechanisms of this weed have been investigated in China, e.g. biological characteristics responsible for its rapid spread, allelopathy on native plants, and effects on soil nutrition and biota. Moreover, we summarize and discuss possible control methods for the crofton weed in this chapter, including chemical control, biological control, replacement control, and comprehensive utilizing.

Keywords Crofton weed • *Ageratina adenophora* • Distribution • Biological characteristics • Allelopathy • Management

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Fig. 25.1 *Ageratina adenophora* invaded in cultivated field

25.1 Introduction

The crofton weed, *Ageratina adenophora* (Sprengel) (Synonym: *Eupatorium adenophorum* Sprengel), native to Mexico and Costa Rica, was introduced into Europe as an ornamental plant in the nineteenth century. Since then, the weed has become invasive in nature and spread rapidly in southern and south-eastern Asia, eastern Australia, New Zealand, and south-western Africa (Gui et al. 2009). Now it becomes a serious weed in many countries, such as Australia, New Zealand, South Africa, Spain, India, Philippine, Malaysia, Thailand, Burma, Vietnam, China, Nepal, Pakistan and pacific archipelago (Wan et al. 2010; Inderjit et al. 2011).

In China, *A. adenophora* was first introduced from Myanmar to Yunnan province in 1940s (Wu and Wang 2005; Gui et al. 2008). Now it can be found in a wide range of habitats, including waste lands, roadsides, cultivated fields, grasslands and forests. *A. adenophora* invades and occupies farmlands, competing with crops for fertilizer, water, sunlight and space, thus causing serious crop harvest losses. It can even dominate the flora of cultivated fields (Fig. 25.1). In addition, it affects livestock production – invasions to grassland may result in heavy losses of native pasture harvest, and exposure to or feeding on the weed can make livestock ill. In China, the annual losses in livestock production due to the effect of *A. adenophora* was estimated to be ¥ 989 million Yuan, and the losses in service function of grassland ecosystem was ¥ 2.625 billion Yuan (Xu et al. 2006; Ding et al. 2007). Furthermore, *A. adenophora* can dominate the flora of the invaded forests (Fig. 25.2), and both the diversity and evenness of species decreased significantly in the coniferous forest, broad-leaved forest, or meadow (Liu et al. 2006; Zhang et al. 2010). The

Fig. 25.2 Forest infested by *Ageratina adenophora*

irreversible reduction in biodiversity threatens native plant species, particularly rare species, ultimately causing serious ecosystem degeneration and altering the local natural landscape. For these reasons, this weed has been ranked as one of the worst invasive alien species in China.

In the past 40 years, extensive researches have been conducted on the biology of *A. adenophora* in China. In particular, great efforts have been put onto the mechanisms underlying in its invasive success. Here we reviewed the research progress on potential invasive mechanisms and major control strategies for *A. adenophora* in China .

25.2 Distribution and Potential Spread of *A. adenophora* in China

Historical invasion pattern and its potential for further spread of *Ageratina adenophora* in mainland China were reconstructed (Lu and Ma 2004, Gui et al. 2008). In 1940s, the earliest populations of *A. adenophora* were found in the southwestern parts of Yunnan province. After that, this weed occurred only in narrow areas in the following 20 years (1940–1960). However, from 1960s it began to spread rapidly north- and eastward in the country at a rate of 20 km on average per year (Wang and Wang 2006). The populations in western Yunnan province spread northward to southern Sichuan province, and further dispersed to the Yangtze River valley in Sichuan province by water flow. Populations in southeastern Yunnan province spread eastward and reached the northwestern part of Guangxi Zhuang Autonomous Region, and spread northward to the central part of Yunnan province and northeastward to the western part of Guizhou Province (Gui et al. 2008). Overall, the weed has been widespread throughout the south and middle subtropical regions of Yunnan, Guizhou, Sichuan provinces, and Guangxi Zhuang Autonomous Region.

Based on the data of dispersal patterns and field survey, *A. adenophora* continues its rapid spread in China (Wang and Wang 2006). Southern and south-central China might be invaded, as the climatic conditions in these regions are favorable for *A. adenophora* growth. However, northern and western China appears unsuitable for this weed.

Methods of geographic information systems and logistic regression have been used to investigate the factors that favor the dispersal of *A. adenophora* (Wang et al. 2011). A number of factors have significantly contributed to the rapid spread of *A. adenophora*. In terms of long-distance dispersal, human activities have probably been the most important factor. Other factors associated with human activities (e.g. frequent ecological disturbance activity along roads and railways) facilitate its invasions over high mountains and across river valleys, providing new populations for further spread. In comparison, natural forces such as wind and water appear to be less important for its dispersal at large geographical scales. However, wind and water can assist the dispersal of *A. adenophora* seeds which are tiny and present with pappus hairs. In addition, wind may play a primary role in local dispersal.

Several eco-geographical factors are involved during the formation of population distribution of *A. adenophora* once they spread into a new region, e.g. elevation, diversity and richness of native plants, man-made disturbance, soil moisture, and so on. Usually, the high abundance and coverage of *A. adenophora* population appear between 1000 and 2000 m elevation, and the coverage of *A. adenophora* was significantly negatively correlated with the coverage of trees and their species richness, and positively correlated with light intensity (Fu et al. 2010). The number of *A. adenophora* seedlings was positively correlated with both the degree of disturbance (e.g. roadsides) and soil moisture in the microhabitats. Scrutiny of characteristics of community structure of various ecosystems revealed that wasteland could become the invasion hotspots, owing to low vegetation diversity and more niche space resulting from overgrazing and higher habitat disturbance. Consequently, dynamic interactions of multiple factors in heterogeneous ecogeographical environments – a 'combinatorial' invasion mechanism would generate an unexpected invasion rate of *A. adenophora* or a seemingly stochastic invasion event (Fu et al. 2010; Zhu et al. 2013b).

25.3 Invasion-Associated Biological Characteristics

25.3.1 Genetic Variation

With the method of inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP), a high level of genetic diversity was detected in *A. adenophora* populations in China (Duan et al. 2005; Huang et al. 2009; Gui et al. 2008, 2009). Populations in southwestern China (Yunnan, Guizhou and Sichuan) showed a high level of genetic differentiation. The estimates of *A. Adenophora* population variation in China, based on ISSR-PCR, were high, as measured by the analysis of molecular variance (AMOVA, $F_{ST} = 0.3140$), the Wright's F-statistics ($G_{ST} = 0.3453$), and the Shannon's information index ($H_{sp} = 0.3716$) (Gui et al. 2009). Genetic differentiation of *A. adenophora* mainly occurred within populations of various provinces. The richness of intra-population genetic diversity was correlated with its colonization time. In Yunnan, the weed possessed the richest genetic diversity and highest genetic differentiation, whereas the population collected from recently colonized areas, such as the population collected in Chongqing, showed the lowest genetic divergence.

Genetic distance was significantly correlated with geographic distance. Genetic diversity of Chinese populations of *A. adenophora* decreased with increased latitude, increasing with the longitude shift from west to east. Genetic diversity of *A. adenophora* was highly positive correlated with the annual mean temperature of its habitat ($R^2 = 0.2731$, $p < 0.01$), while non-correlated with annual mean rainfall (Gui et al. 2006).

25.3.2 Seed Banks and Germination Characteristics

Plants that have persistent seed banks in soil would have advantages to pass through stress and harsh environments. *A. adenophora* has a persistent soil seed bank with a wide range and high density in invaded areas (Shen and Liu 2004), e.g., in bare land habitat, the annual seed rainfall of *A. adenophora* amounted to 2.4×10^5 individuals per square meter and all the seeds were distributed in the soil at a depth of 2 cm. Furthermore, maximum emergence occurred when seeds were deposited on the soil surface, and sandy loam soil was much favorable for seed germination (Wang et al. 2006).

Seeds of *A. adenophora* can germinate under an abroad rang of temperatures (10–30 °C). The crofton weed was moderately photoblastic, with only 17% germination occurring in the dark. A very high germination rate (94%) was observed in distilled water at pH 5.7 (Lu et al. 2006). Thus, possible prevention and control measures to *A. adenophora* should be based on the characteristics of seed bank and germination. For example, *A. adenophora* was found with one feature of photoblastic seed germination, which plays a key role in forming soil seed bank and facilities the fast-invasion of this weed in disturbed soil, where seeds in deep soil come to surface after disturbance (Jiang et al. 2013a). The ecological control of this weed by introducing suitable trees to influence light environment around it should be available.

It is said that if some of the biological characteristics give priority to the invader than the native congeners of invader, then these factors may play a role in invasion success (Inderjit et al. 2008), and this always is called the congeneric comparative methods. When compared with native congeners (*E. japonicum*, *E. chinense*, *E. stoechadosmum*) in terms of seed morphological traits and germination associated with environmental factors, e.g. temperature, *A. adenophora* was significantly lower in seed weight, length, width, and pappus length. Seeds of *A. adenophora* germinated in a wider range of temperatures than *E. japonicum* and *E. chinense*, and in a sub-hypothermia, the invader had higher germination ratio and germination index than the two natives (Zhang et al. 2014). These traits mentioned above may facilitate invasion success of *A. adenophora*, which will be helpful to explore the mechanisms underlying its invasiveness from the contemporary evolution point.

25.3.3 Nitrogen Reallocation and Quicker Return Energy-Use Strategy

The increased vigor in invasive populations is thought to be due to life history tradeoffs, in which selection favors the loss of costly defense traits, thereby sparing resources that can be devoted to increased growth or fecundity (Feng et al. 2009). When compared with their native populations, the invasive populations of *A. adenophora* appear to have evolved to increase N allocation to photosynthesis and reduce allocation to cell walls, resulting in poorer structural defenses (Feng et al. 2009). This finding demonstrates a potential mechanism behind the commonly observed and genetically based increase in plant growth and vigor when they are introduced to new ranges.

Moreover, invasive populations (China and India) of *A. adenophora* were found to grow faster with significantly higher leaf nitrogen concentrations and specific leaf areas than the native populations (Mexico) (Feng et al. 2011). It was found that populations of *A. adenophora* in non-native ranges had a distinct quick return energy-use strategy, with high photosynthetic energy-use efficiency and a short payback time but not lower construction costs. These traits showed us the possible mechanisms underlying in the commonly observed increase in growth when plants are introduced to new habitats.

25.3.4 Adaptation to Environmental Stresses

Light could be an important factor affecting the growth and distribution of *A. adenophora*. Zhu et al. (2013b) investigated the growth characteristics of *A. adenophora* under different manmade forest communities for three years. They found that the density, plant height, branches, buds and seeds of this weed were positively correlated with light intensity. Interstingly, *A. adenophora* can dissipate excessive light energy efficiently under high light, whereas it can absorb light energy sufficiently under low light. This allows *A. adenophora* to utilize light energy effectively and grow normally in a wide range of light intensity (Wang et al. 2004). Furthermore, *A. adenophora* has typical leaf morphological adaptation to different light conditions (Sun et al. 2006). At low light levels, plants would enhance light availability by increasing biomass allocation to leaves and forming larger, thinner leaves with high specific leaf area, leading to a high leaf area ratio and high stem strips length. The growth of seedlings at 30–55% relative irradiances was much better than that at full light condition. This might be an adaptive strategy that supports the vigorous invasiveness of this species, because a high-shaded canopy could prevent other plant species from surviving and growing. For example, when compared with two native congeners (*E. heterophyllum* and *E. japonicum*) at different irradiances, *A. adenophora* had a higher light-saturated photosynthetic rate (Pmax) and total leaf area, which may contribute to its higher relative growth rate (RGR) and total biomass

(Zheng et al. 2009). The higher support organ mass fraction and the lower root mass fraction of *A. adenophora* may also contribute to its higher RGR, and through increasing carbon assimilation and reducing respiratory carbon loss to contribute its higher biomass. Thus, the higher growth rate of the invader increased its total leaf area, ramet number, and crown area.

A. adenophora from different populations in invaded regions showed adaptability to other environmental factors, such as drought (Su et al. 2005a; Zu et al. 2005), heat (Su et al. 2005b), and elevated CO_2 concentration (Xiao et al. 2014). Thus, genetic differentiation and plasticity of *A. adenophora* may give it assistance to invade heterogeneous habitats (Zhao et al. 2012). For example, Li and Feng (2009) compared seed morphometric and germination traits among 14 populations of *A. adenophora* located at different elevations in south Yunnan Province. They found that large seeds, high germination capacity, and high germination index could improve emergence, establishment, growth, and survival of seedlings at high elevation; the low germination index could prevent seed germination before the onset of the rainy season at low elevation. Investigation on CO_2 exchange characteristics indicated that *A. adenophora* had a large variability in photosynthesis and respiration when grown in different habitats and at different stages of growth (Wang et al. 2005). Meanwhile, under elevated CO_2 concentration, *A. adenophora* evidently promoted biomass accumulation and photosynthesis without a decrease in chlorophyll, and such promote was not affected by the depletion of NH^{4+} (OuYang et al. 2014). Thus, *A. adenophora* might be more competitive in areas where the soils are relatively poor in NH^{4+} as levels of atmospheric CO_2 continue to rise.

25.4 Allelopathy on Native Plants

Several studies suggest that allelopathy has helped *A. adenophora* to become dominant in invaded plant communities (Yang et al. 2008). Foliar leachates and volatiles of this plant could decrease the seed germination and seedling growth of some native plant species, e.g. *Brassica rapa*, *Chlorisg ayanas*, *Ixeridium gracile*, *Macrothelypteris torresiana*, and *Mariscus cyperinus* (Song et al. 2000; Yu et al. 2004; Zheng and Feng 2005; Zhang et al. 2008, 2012). Meanwhile, *A. adenophora* may be affected by autotoxicity (Zhu et al. 2014). Field experiments showed that emergence of *A. adenophora* seedlings was significantly increased when the field was deprived of dry branches, fallen leaves and parental plants (Yu and Ma 2006). Addition of activated carbon into the soil infested with *A. adenophora* may also alleviate autotoxicity (Tian et al. 2007).

The allelopathic potential of *A. adenophora* varied owing to some factors, such as development stage, growth site, and some stresses. Han and Feng (2007) indicated that allelopathy of *A. adenophora* from the aquatic extract of leaves and stems on native plants increased with developmental stage. The allelopathy from aquatic extract of *A. adenophora* growing along roadside was found to be stronger than that from the plants growing under evergreen broad-leaved forests and deciduous-broad leaved forests. Moreover, its allelopathy was significantly correlated with the relative abundance of native species (Yu et al. 2004). Interestingly, treated with leaves holing and plants cutting could increase the contents of some allelochemicals significantly (Yang et al. 2014). Meanwhile, soil microbial communities could regulate interactions between *A. adenophora* and native species by breaking down the allelochemicals excreted by the invader (Zhu et al. 2011).

Some putative allelochemicals of *A. adenophora* have been identified, such as limonene, 2-carene, a-pinene and camphene from volatile (Zhang et al. 2012), 3-(2-hydroxyphenyl) propyl methyl malonate, 3-(2-hydroxyphenyl)-1-propanol, and *o*-coumaric acid from the extract of root (Zheng et al. 2012; Zhou et al. 2013), dis(2-ethylhexyl) phthalate (DEHP), eupatorenone, dibutyl phthalate (DBP), amorpha-4,7(11)-dien-8-one (DTD) from the root exudates (Yang et al. 2013), and DTD and 6-hydroxy-5-isopropyl-3,8-dimethyl-4a,5,6,7,8,8a- hexahydraphthalen-2(1H)-one (HHO) from the leachates (Yang et al. 2006). The putative allelochemicals degraded rapidly in the soil, and the low levels of allelochemicals observed in the soil may be sufficient to affect seed germination and plant growth (Yang et al. 2016).

25.5 Effects on Soil Nutrition and Biota and Their Feedback

Plant invasions may alter soil nutrition and underground microbial communities, which, in turn, may affect the interaction between invasive plants and resident native species. Growing evidence suggests that feedback of soil biota to invasive and native plants may lead to successful exotic plant invasions (Inderjit and van der Putten 2010). Invasion of *A. adenophora* would increase the contents of several chemicals in soil, such as nitrate nitrogen (NO^{3-}-N), ammonium nitrogen (NH^{4+}-N), and available P and K (Niu et al. 2007).

A. adenophora invasion strongly increased the abundance of vesicular-arbuscular mycorrhizal fungi (VAM) and the fungi/bacteria ratio in soil (Niu et al. 2007). The soil biota in the heavily invaded site had a greater inhibitory effect on native plant species than on *A. adenophora* itself. Soil biota alteration after *A. adenophora* establishment might be an important part of its invasion process to facilitate itself and inhibit native plants. Recent studies found that *A. adenophora* invasion significantly increased the number and diversity of nitrogen-fixing bacteria in soil (Xu et al. 2012). Meanwhile, *A. adenophora* could hearten arbuscular mycorrhizal fungi (AMF) in rhizosphere soils and induce positive AMF feedback, which enhanced *A. adenophora* invasiveness (Yu et al. 2011).

25.6 Sustainable Management

25.6.1 Chemical Control

The control efficacy of some herbicides on *A. adenophora* at different growth stages was evaluated (Liu et al. 2011; Zhu et al. 2013a). Paraquat, glufosinate, picloram and garlon showed effective control on *A. adenophora*. Paraquat and glufosinate can kill the weed fast with short duration of efficacy but the efficacy of picloram and garlon lasted for 360 days after the herbicide application while maintaining the efficacy at 90%. The control efficacy of sulfometuron-methyl 315–630 g•hm^{-2} and saflufenacil 157.5 g•hm^{-2} were also over 50% after 90 days of herbicide application. Sekator did not show good control efficacy and MCPA showed little control efficacy towards *A. adenophora*. Flowering and fructification of *A. adenophora* were strongly inhibited after application of sulfometuron-methyl 315–630 g•hm^{-2} and saflufenacil 157.5 g•hm^{-2}. Application of sulfometuron-methyl and saflufenacil was suggested to provide good ecological control in heavily invaded and proliferation regions of *A. adenophora*. Moreover, herbicides provided a better efficacy at the vegetative stage compared to reproductive stage of *A. adenophora*. Although some kinds of herbicides can be used to control *A. adenophora* effectively, the use of chemical control must be carried out wisely to avoid the possible negative effects to the environment. So, some dose-saving methods were also explored, such as picloram 10% ED when used with electrostatic spraying on *A. adenophora* attained 20% higher control effect than that of picloram 24% AS at the same dosage (Liu et al. 2011); half dose of recommended of sulfometureon methyl and picloram could inhibit photosynthesis of *A. adenophora* effectively (Liu et al. 2010).

25.6.2 Biological Control

Classical biological control is one of the most effective approaches for controlling alien invasive species (Wan et al. 2008). In the past decade, some biological control agents have been evaluated for their control efficiency against *A. adenophora*, such as the fungus, *Alternaria alternata* (Fr.) Keissler Strain 501 (Qiang et al. 2002, 2006). The authors found that *A. adenophora* seedlings at the four-leaf-pair stage were more susceptible than the older plants, and suggested that mycelia be more suitable as infection propagules for *A. alternata* strain 501 in a bioherbicide for the control of *A. adenophora*. Further investigation indicated that the mode of the toxin extracted from the isolate of *A. alternate* inhibited photosynthesis through electron transfer reaction of two photosystems (Chang et al. 2004; Dai et al. 2004).

The imported tephritid gall fly, *Procecidochares utilis* Stone (Tephritidae, Diptera) (Fig. 25.3), was introduced into Yunnan Province in 1984 (Dai et al. 1991). The plant height, joint number, total leaf area, and photosynthesis of *A. adenophora* reduced significantly after being parasitized by *P. utilis* (Zheng et al. 1989)

Fig. 25.3 Adult of *Procecidochares utili*

(Figs. 25.4–25.5). Chen and He (1990) reported that it was optimal that one *P. utilis* held ten shoots of crofton weed for its releasing on field. However, *P. utilis* was also reported as a sublethal factor, instead of obvious harmful impact on the growth and reproduction of the crofton weed (Li et al. 2006). In addition, the methods of releasing *P. utilis* and the technique of mass rearing of *P. utilis* were advanced in China (Wei et al. 1989; Yang 2010). Parasitism rate by the *P. utilis* was significantly affected by habitats, higher in open fields (55.4%) than under forest trees (41.2%).

Furthermore, one native insect *Orthezia quadrua* was reported as as natural enemy to *A. adenophora* (Xu et al. 2011). This insect gathers on the node of stem in general, and sucks plant juices. At present, using biological control agents can only decrease the dispersal speed of the crofton weed, and unfortunately could not eliminate this weed completely.

25.6.3 Replacement Control **Setaria sphacelata**

The replacement control, based on the competitive phenomenon among various plant species, has been used to inhibit the growth of the crofton weed (Sun et al. 2004). Such a method could be used in the areas for forestry and animal farming (Fig. 25.6). The plants chosen for replacement should be easy to be grown, have a high economic value, and can reach a high canopy density within a short period of time. In the past decade, a number of greenhouse or field experiments were conducted in China to evaluate the root competitive capacity of replacement plants against *A. adenophora*. Several weeds have been demonstrated as ideal candidates of replacement plants, such as *Trifolium repens*, *Trifolium pratense*, *Pennisetum hydridum* (Sun et al. 2004), *Setaria yunnanensis*, *Eupatorium fortunei*, *Chenopodium serotinum* (Jiang et al. 2014), Grass, e.g. *Setaria sphacelata*, *Imperata cylindrica* (Peng et al. 2010a, b), *Pennisetum clandestinum* (Dong et al. 2006). Moreover,

Fig. 25.4 Stem of *A. adenophora* infected by *Procecidochares utilis*

some fast-growing trees possessing allelopathic potential again *A. adenophora* were also identified, e.g. *Eucalyptus citriodora*, *Acacia confusa* (Sun et al. 2004; Xia et al. 2013), *Camptotheca acuminata* (Zu et al. 2007), and *Eucalyptus globulus* (Huang et al., 2007).

Interbreeding of multi-pastures is a potential way for the replacement control of *A. adenophora*. Among these candidates plants described above, *S. sphacelata* was the most effective one. When planted together, *S. sphacelata* has a higher relative competitive ability than *A. adenophora*. *S. sphacelata* could substantially decrease the growth of *A. adenophora*, and such inhibiting effect increased as the density of *S. sphacelata* increased. When the proportion of planted *S. sphacelata* to *A. adenophora* reached 3:1, biomass of *A. adenophora* could be reduced by over 80%. Field experiments over a few years showed that *S. sphacelata*, when planted at the density of 45 m^{-2}, could out-compete and replace native plants, and thus prevent *A. adenophora* from re-colonization after being uprooted. Additionally, rhizophere soil microbes of *A. adenophora* in mixed-cultured area was remarkably higher than those in the invaded mono-culture area. Therefore, *S. sphacelata*, as a competitor, is a promising candidate for replacement control of *A. adenophora*.

25.6.4 Comprehensive Use of A. adenophora

The use of *A. adenophora* has been explored in past several years. The extract of *A. adenophora* was tested for its control efficacy again some insect pests in greenhouse or fields, e.g. *Helicoverpa armigera* (Cheng et al. 2007), *Panoychus citri* (Li et al. 1995), *Aphis gossypii* (Wang et al. 2002), and weevils (Li et al. 2001). The plant extract of *A. adenophora* has also been used to produce some valuable natural chemicals, such as xylitol by microorganism fermentation (Yang and Li 1999),

Fig. 25.5 Larval of *Procecidochares utilis* at the stem of *A. adenophora*

methane by anaerobic fermentation (Zhang and Yang 1997; Chen et al. 2014), and cellulose (Jiang et al. 2013b).

25.7 Conclusion and Perspectives

The invasion success of *A. adenophora* is the result of interaction of its bio-characteristics and the susceptibility of invaded habitats, and the crofton weed may develop adaptive evolution to adapt to diverse environments. Available evidence suggests that *A. adenophora* in China have developed considerable genetic diversity by admixture of mutilple invade sources, with various populations forming relevant adaptive evolutions, such as adaptive shape change and modification of characteristics. *A. adenophora* has formed adaptabilities to environments in the invaded areas during the process of invasions, which were gradually enhanced over time, and widened distribution areas of the weed caused more negative effects on ecosystems. Meanwhile, the inhibitory effect of allelochemicals from *A. adenophora* on native plants is also an important factor during its expansion. *A. adenophora* modified soil microbial communities in invaded areas, which strengthened its growth and competition with native plants, but this phenomena needs further investigation concerning other invasive weed species. Further studies are needed to investigate the invasion

Fig. 25.6 Replacement of *Ageratina adenophora* using *Setaria sphacelata*

mechanisms, effects on structure and function of invaded communities, establishment, development, potential harmfulness and integrated management of *A. adenophora*.

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Chapter 26
Mile-a-Minute Weed *Mikania micrantha* Kunth

Shichou Han, Zhigang Li, Qiyun Xu, and Lingling Zhang

Abstract The mile-a-minute *Mikania micrantha* Kunth is native to tropical Central and South America. In China, this species is mainly distributed in Southern China, such as Hong Kong, Yunnan, Taiwan, Guangdong, Hainan, Guangxi, Macao and Jiangxi. *M. micrantha* causes native plants which are unable to photosynthesis thereby then suppresses their growth even kills them. The weed has caused disastrous damages to various agricultural and forestry ecosystems in China, including farmlands, orchards, forests, and so on. A number of economic trees such as oil palms, coconuts, cocoa, tea trees, rubbers and teaks have suffered serious damages from this weed, resulting in huge economic loss. In the past two decades, enormous studies have been conducted on this weed regarding its control approaches, including mechanical removal, chemical control, biological control, and ecological control. In this chapter, we reviewed the major information of these aspects and present the prospective on what would have to be done to develop an integrated management system for this weed.

Keywords *Mikania micrantha* • Distribution • Damage • Biological control • Natural enemies

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26.1 Introduction

The mile-a-minute weed *M. micrantha* Kunth (Asteraceae), a rapid-growing, perennial, semi-woody vine, is one of the top ten worst weeds and 100 most invasive alien species in the world (Lowe et al. 2001; Wu et al. 2013; Prabu et al. 2014). Native to tropical Central and South America (Zhang et al. 2004), this weed has spread to tropical Asia, Papua New Guinea, Indian Ocean islands, and Pacific Ocean islands (Zhang et al. 1977, 2004; Manrique et al. 2011). *M. micrantha* is mainly distributed in the areas with an altitude below 200 m, especially in valleys and river side's.

In China, it has invaded various ecosystems, including farms, natural lands (e.g., margins of forests and wetlands), roadsides, and abandoned farmlands. The areas planted with tea, fruits, rubber and teak can be seriously damaged, causing huge economic losses and ecosystem problems (Zhang et al. 2004; Zan et al. 2000; Li et al. 2007; Yu et al. 2011). The resulted economic loss in natural areas was estimated to be more than several hundreds of millions of US dollars per year (Zhong et al. 2004). For this reason, *M. micrantha* was listed as one of 16 serious exotic invasive species in 2003 by Ministry of Environmental Protection, and as one of 21 forestry quarantine pests by State Forestry Administration in China (Wang et al. 2013).

26.2 The Distribution and Damage of *Mikania micrantha* in China

26.2.1 Geographic Distribution

In mainland China, *M. micrantha* was first recorded in 1884, when it was cultivated at Hong Kong Zoological and Botanical Gardens (Zhang et al. 2004). It was naturalized during the following 35 years, and began to expand rapidly into other regions outside Hong Kong since 1950s (Zhang et al. 2004). Now, the weed has widespread in Southern China (Zhang et al. 2004; Fan et al. 2010), such as Guangdong (Wang et al. 2003, 2013; Lian et al. 2014), Yunnan (Du et al. 2006; Shen et al. 2012), Hainan (Fan et al. 2010; Peng et al. 2013), Guangxi (Guo et al. 2013) and Jiangxi (Zeng 2013).

26.2.2 Damage of **Mikania micrantha**

M. micrantha has a great breeding and climbing ability. It can crawl on the ground, twine and cling onto its nearby plants, and climb upward on tall tree branches (Fig. 26.1). As a result, once this weed establishes most of underneath shrubs and nearby trees may be covered at the crown, growth is seriously suppressed, and finally is killed due to reduction in available light (Zhang et al. 2004; Hu and Li

Fig. 26.1 *M. micrantha* clings onto nearby plants

2014). The water and nutrients available to native plant may also be reduced. For these reasons, *M. micrantha* has become one of most serious invasive plants in farmlands, orchards, and forests in China (Xu et al. 2013; Zhang et al. 2004). A number of crops and trees have been seriously affected, including oil palms, coconuts, cocoa, tea trees, rubbers, and teaks. The economic loss resulted from *M. micrantha* is huge. For instance, in the Pearl River Delta of China, the estimated costs were as high as 500 million RMB to 8 billion RMB every year (Peng et al. 2013). The Pearl River Delta has become the most seriously damaged region in China.

Moreover, *M. micrantha* may affect structure and functioning of ecosystems in the invaded areas. It can alter soil properties, affect nutrient cycling, and alter or reduce local biodiversity (Hwang et al. 2003; Zhang et al. 2004; Wang et al. 2004; Kaur et al. 2012; Wu et al. 2013). Functions of invaded forest ecosystem would be weakened with respect to water conservation, CO^2 fixing, and so on.

26.3 Factors Contributing to *Mikania micrantha* Spread in China

Several intrinsic features of this weed have contributed to its rapid spread in China. They have a high reproductive capacity and growth rate, great morphological plasticity (Li et al. 2012), high compensation capacity (Lian et al. 2006), and rapid adaptive evolution (Wang et al. 2008; Huang et al. 2015b). It can also adapt to a number of environmental stresses, such as acid rain (Cao et al. 2010).

M. micrantha is reproduced both sexually and asexually. Biomass of the flowers amounts to 38.4–42.8% of the total above-ground plant biomass (Hu and Paul 1994), thus it can produce a huge number of seeds annually. It can also be reproduced by rapid propagation of vegetative parts. The stem nodes and internodes may develop into new plants, and each section of the leaf axils can grow into new branches. Thus, this weed has a high regeneration capacity after fragmentation (Li et al. 2013; Huang et al. 2015a).

Moreover, *M. micrantha* plants, residues, and even the soil beneath the plant stand have allelopathic effects, which may inhibit seed germination and seedling growth of native plants (Ismail 2002; Wu et al. 2009; Li and Jin 2010). Due to this reason, it can outcompete successfully over native plants.

26.4 Management of *Mikania micrantha*

In the past two decades, enormous researches have been conducted on this weed regarding its control approaches, including mechanical removal, chemical control, biological control, and ecological control (Zhang et al. 2004; Kuo et al. 2002; Yu et al. 2009; Shen et al. 2013).

26.4.1 Biological Control

26.4.1.1 Investigation of Local Natural enemies

Han et al. (2001) and Shao et al. (2002) investigated the arthropods and diseases on *M. micrantha* in Guangdong Province from 2001 to 2002. They collected 58 arthropod species which belonged to 43 families of 15 orders. Of them, the aphid *Aphis citricolor* and mite *Eotetranychus* sp. were most abundant. They were reproductive highly during some periods of the seasons; however, they are omnivorous insects and thus could not cause death of the weed.

Chen et al. (2003) reported that the mite *Brevipalpus phoenicis* (Geijskes) could feed on and inhibit the growth of *M. micrantha*. However, this enemy appeared to have little potential of being a biocontrol agent, because it was unable to complete life cycle when the average temperatures was over 30 °C or below 20 °C. Ze et al. (2013) reported that one stinkbug *Pachypeltis* sp. obligatively fed on *M. micrantha*. The stinkbug had strong feeding ability and big appetite, and was capable of suppressing stem growth, clone generation, inflorescence differentiation, flowering and seeding quantity of the weed. Thus, this enemy can be expected to control the weed effectively.

26.4.1.2 Introductions of Natural Enemies–Two Butterflies

Butterflies *Actinote anteas* and *A. thalia pyrrha* (Nymphalidae, Lepidoptera) were discovered by Mcfadyen in the country of origin, Costa Rica, in 1967. In 1996, Dr. R. Desmier worked in Indonesian oil palm Research Institute from Costa Rica, introduced these two butterflies and developed the technique for laboratory breeding and field releasing. So far, both butterflies have already established natural populations in low-elevation hills in Indonesia. In 2001 and 2002, Guangdong Entomological Institute successively introduced the two butterflies from Indonesia Oil Palm Research Institute for the control of *M. micrantha* (Li et al. 2002b). Then, host specificity of *A. anteas* was tested using 83 plant species belonging to 40 families in Guangdong (Li et al. 2004). The results revealed that this butterfly completed life cycle only on *M. micrantha* and another alien invasive plant in China, *Chromolaena odorata* L.

Now, rearing technique has been developed to propagate the butterflies successively both in the laboratory and wild, using cultivated *M. micrantha* plants. However, the two butterflies cannot adapt to high temperature of the summer in Guangdong, thus their population growth capacity is low under natural conditions.

26.4.1.3 Introduction of the Rust Fungus *Puccinia spegazzinii*

P. spegazzinii is a classical biological control agent for *M. micrantha* (Ellison et al. 2008). In 2003, the UK Centre of CABI (Centre for Agriculture and Biosciences International), Guangdong Entomological Institute and Institute of Environment and Sustainable development in Agricultural, Chinese Academy of Agricultural funding for biological control *M. micrantha* in China under the Darwin Initiative, obtained the approval from relevant departments and introduced some rust strains from UK. The host specificity of the fungi was tested on 72 plant species which belonged to 29 families, 62 genera under the condition of isolation. The results revealed that the fungi successfully colonized on *M. micrantha* and *M. cordata*, and also formed chlorotic spots on several other plant species including *Asparagus cochinchinensis*, *Ageratina adenophora*, *Helianthus annuus*, *Elephantopus scaber* L. (Fu et al. 2006). *P. spegazzinii* has several promising attributes, such as high host specificity and high capacity of causing death in host plants. On the other hand, as *P. spegazzinii* requires a high humidity for spore germination and propagation which normally cannot be met under natural conditions, its control efficiency is generally low.

26.4.2 Ecological Control

Ecological control, such as using replacement control technology based on relationships between invasive and native plants (e.g., parasitism and competition), represents a promising method for controlling alien invasive plants. The research progress in the control of *M. micrantha* is as below.

26.4.2.1 Use Dodder for Controlling *M. micrantha*

Dodders are important parasitic plants of *M. micrantha* (Han et al. 2002). They wrap around the weed's stems and absorb nutrition by sucking device from there. In Southern China, there are three dodder species that can affect *M. micrantha*, i.e., *C. campestris* Yunker, *C. chinesis* Lam, and *C. australis* R. Br. To clarify the control efficiency of various dodders, a 5-year comprehensive investigation was conducted over hundreds of acres in Shenzhen, Dongguan, Shanwei, Hongkong, where *C. campestris* was determined as a dominant parasite. Field surveys revealed that *C. campestris* often flourished in the place where *M. micrantha* grow. Death of *M. micrantha* due to parasitism of this dodder could often be observed in the fields. Thus far, *C. campestris* has been considered as a potential biocontrol agent of *M. micrantha*. In addition, it was also found that *C. campestris* is unable to eliminate underground part of *M. micrantha* completely, thus the two plants often coexist with the dodder.

26.4.2.2 Community Restructuring

M. micrantha grows well in an open environment, but it poorly grows or cannot grow under shaded conditions (Ipor 1991; Wen et al. 2000). As indicated by Yang et al. (2003), the weed was rarely distributed in the canopy with a covering of over 60% under forest environments. Seed germination, plant growth, flowering and fruiting of the weed could be greatly affected by light (Hu and Paul 1994; Li et al. 2002a; Zhang et al. 2002; Yang et al. 2003). Thus, closed environments are not suitable for the weed. Based on these findings, several field trials have been conducted to find suitable tree species for use in community restructuring of *M. micrantha* invaded ecosystems. For example, Yin et al. (2003) found some plants that were capable to suppress *M. micrantha* growth, such as *Acacia auriculiformis*, *A. mangium*, *Mytilaria laosensis* and *C. camphora*. These plants grow faster at the canopy than *M. micrantha*, thereby inhibiting the invasions of the weed in a short term. Yu (2004) reconstructed a community in Neilingding Island, Shenzhen, using a number of tree species, such as *Macaranga tanarius*, *Syzygium hainanense*, *Heteropanax fragrans*, *Cinnamomum burmannii* and *Podocarpus nagi*, for the purpose of suppressing the weed.

More promising results came from a community-restructuring experiment in Shenzhen, conducted in 2008 by Shenzhen wildlife management service partnership and Professor Li Mingguang from the Sun Yat-sen University. They showed that, after 5 years, the selected tree species in experimental plots grew well, and the canopy was continuing to grow, which substantially reduced the illumination under canopy. In this experiment, *M. tanarius* and *H. fragrans* were the most promising tree species to control the weed, followed by *C. burmanii* and *Endospermum chinense* Benth. After that, a community restructuring practice was implemented in Huidong Street Green village by Prof. Li and Huidong County Forestry Bureau. The damage of the weed in this area has been effectively controlled.

Recently, the mechanism responsible for the suitability of native plants for community restructuring was investigated. For example, Gao et al. (2013) found that soil fungi of native tree species might contribute. Such microbes could inhibit biomass production and shift biomass allocation of invasive *M. micrantha*.

26.4.3 Chemical Control

The use of chemicals is a major method for the control of *M. micrantha* in large areas. In China, sulfometuron-ethyl, glyphosate, sulfometuron methyl and mieweijing are the main chemical herbicides for the control of this weed (Xu 2001; Zan et al. 2007; Zhao and Liu 2012). Among them, sulfometuron-ethyl and glyphosate have been widely used in China. However, they have to be used with particular cautions, otherwise other plants and crops might be damaged. For example, many groups of plants, e.g., Compositae, Gramineae, Moraceae and cruciferous plants are highly sensitive to sulfometuron-ethyl. In addition, glyphosate has the shortage that it cannot kill the roots of the weed. Mieweijing (18% 2, 4-D ME) has a selective specificity for *M. micrantha* and thus basically would not affect other weeds. But it may damage fruit trees. Collectively, safe and effective chemicals are still high needed to control this weed in China.

26.4.4 Manual Removal

Muniappan and Viraktamath (1993) reported the artificial removal of *M. micrantha*. In China, Zan et al. (2000) removed 2000 m^2 of the weed by manual removal in the Neilingding Island. They cut out the ground vegetation and invisible roots and stems prostrate on the ground, and exposed the weed in the sun. However, after 6 months, the weed sprouted quickly due to the failure of drying stem sections in the rainy season. To control the weed, Guo et al. (2002) cut the vines three times during the summer and autumn, and they achieved a better result. Overall, manual removal is expensive due to high labor cost, and it can be used only in small areas.

26.5 Conclusions and Perspective

So far *M. micrantha* has spread to Southern China, such as Hong Kong, Yunnan, Taiwan, Guangdong, Hainan, Guangxi, Macao and Jiangxi. The Pearl River Delta region has particularly suffered from serious damage. At present, various management strategies, including biological control, ecological control, herbicides application, and manual removal, have been used to control this weed.

In the past 20 years, there has accumulated many successful control experiences. However, further research efforts are needed. (i) We should strengthen the risk assessment of this weed. The areas that have not been invaded by this weed are to be monitored. (ii) Some biological agents have to be further evaluated for their control efficiency, and be improved of their rearing and releasing technique, such as the native mite *B. phoenicis* and the introduced butterfly *A. anteas* and rust fungus *P. spegazzini*. (iii) International exchange and cooperation is needed to be strengthened, and information databases are to be established to make full use of the data of this weed. (iv) The use of *M. micrantha* as a resource for ecological and economic purposes is to be exploited. This includes the use of this weed to absorb heavy metals in soil, and to develop culture media of insects and microbes, drugs for humans and animals (e.g., anti-tumor and antimicrobial drugs), and fertilizers. Overall, as no single control method can effectively reduce the damages caused by *M. micrantha*, an integrated management strategy has to be developed in China.

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Chapter 27
Canada Goldenrod *Solidago canadensis* L.

Fengjuan Zhang and Fanghao Wan

Abstract The Canada goldenrod *Solidago canadensis* L. is a rhizomatous, patch-forming herbaceous perennial plant in the family Asteraceae. It was introduced into China in 1935. In addition to its prolific vegetative propagation, *S. canadensis* releases chemicals that inhibit the growth, germination and survival of native plants, and change the soil composition by diverting nutrients and minerals. Soil water content has greatly influenced the growth of this invasive plant. It is currently abundant in four provinces of southeastern China, with scattered populations in other provinces. The latitudes of the potential range span from 25° to 50°. *S. canadensis* has caused great ecological threat to biodiversity. Chemical control, mechanical eradication and biological control are possible strategies for management of this invasive plant. Besides, *S. canadensis* contains a wide range of bioactive compounds that are responsible for its antioxidant, antimicrobial, anti-inflammatory and spasmolytic and diuretic properties.

Keywords *Solidago canadensis* • Alleopathy • Distribution • Ecological adaptability • Control methods

27.1 Introduction

The Canada goldenrod *Solidago canadensis* L. is a rhizomatous, patch-forming herbaceous perennial plant in the family Asteraceae (Werner et al. 1980) (Fig. 27.1). This plant is native to North America and has become a successful invasive species since its introduction to Europe in the eighteenth century (Semple and Cook 2006). So far, it has successfully invaded Europe, large parts of Asia, Australia and New Zealand (Weber 1998; Lu et al. 2007). *S. canadensis* seriously affects the growth of

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Fig. 27.1 *Solidago canadensis* L.

other plants, mainly owing to its super competitive ability for nutrients, water and space, releasing chemicals to inhibit the growth of native plant species (Yang et al. 2007a, b; Abhilasha et al. 2008) and its prolific vegetative propagation (Meyer and Schmid 1999) . Moreover, it can also change soil composition by diverting nutrients and minerals (Zhang et al. 2009a), which is beneficial to its invasions.

27.2 Invasion History and Current Distribution of *S. canadensis* in China

S. canadensis was introduced into China as an ornamental plant in 1935 (Lu et al. 2007; Li and Xie 2002). But from early 1980s, this plant began to spread rapidly (Guo and Fang 2003). Since 2000 it has become one of most serious invasive alien species in China, colonizing a wide range of wilds and disturbed habitats.

The spread of this alien weed is very rapid in China, already widespread in four provinces of southeastern China, i.e., Shanghai, Jiangsu, Zhejiang and Anhui. Besides, it has also been found in western and northern China (Lei and Xu 2010). *S. canadensis* has the high potential to invade other regions. According to Lu et al. (2007), northeastern China might be suitable for *S. canadensis* growth, and a wide range from 25 to 50°N might be occupied by this plant (Lu et al. 2007; Xu et al. 2014) (Fig. 27.2). Intensity of agricultural activities was considered to be one of the major factors causing its rapid spread in this country (Chen et al. 2009).

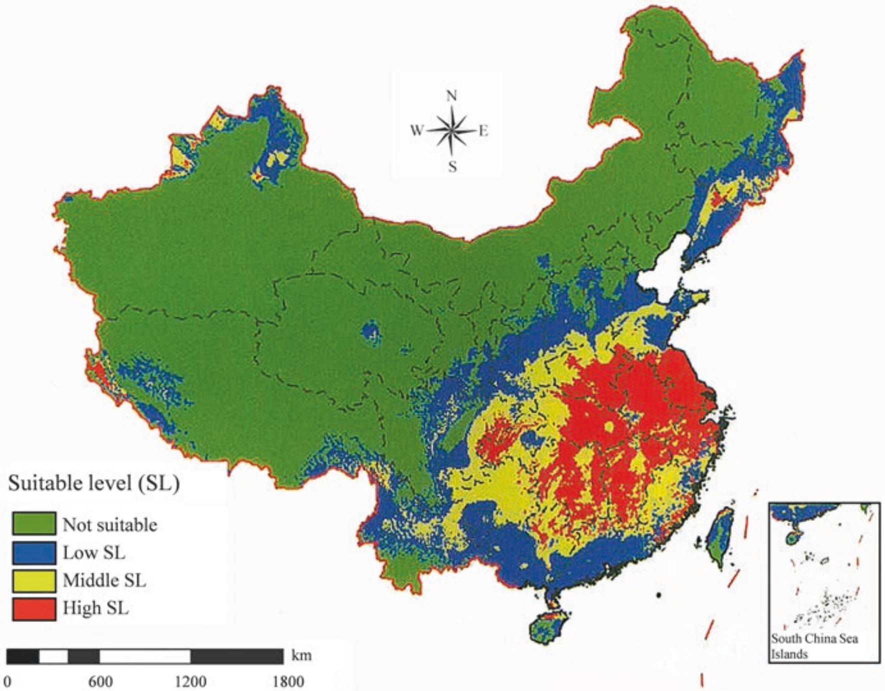

Fig. 27.2 The potential range of *Solidago canadensis* in China (Lei and Xu 2010)

27.3 Biological and Ecological Properties

27.3.1 Allelopathic Effects of S. canadensis

S. canadensis is a highly invasive plant. It can reduce local species diversity or locally out-compete all native plants once established. Its invasion success is due to the release of allelopathic compounds from this plant (Yang et al. 2007a; Abhilasha et al. 2008; Zhang et al. 2012).

During the invasion process, *S. canadensis* has evolved to be more allelopathic, which in turn has greatly favored the increasing of its competitiveness in introduced ranges (Yang et al. 2007a, b; Yuan et al. 2012; Wang et al. 2016). Zhang et al. (2014) studied the effect of 4-pathways of allelochemicals release on wheat (*Triticum aestivum* L.) and radish (*Raphanus sativus* L.). They found that leachates from stems and leaves and decomposed residue of *S. canadensis* were allelopathic to seed germination and seedling growth. Phenolics, n-hexadecanoic acid, flavones and saponins were its major allelochemicals. *S. canadensis* plants sampled from China had greater allelochemical contents and allelopathic effects than those from the USA (Yuan et al. 2012; Sun et al. 2006; Li et al. 2011; Zhang et al. 2007a).

S. canadensis had significant influence on the function and structure of soil microbial communities, and the changed microbial communities tended to benefit themselves (Dong et al. 2015; Zhang et al. 2009b; Liao et al. 2011, 2013). Aerobic bacteria, fungi, actinomycetes and the ratio of fungi PLFAs to bacteria PLFAs significantly increased when *S. canadensis* became dominant (Liao et al. 2013). Its invasion changed the dominant arbuscular mycorrhizal fungal (AMF) species in its rhizosphere soil. For example, Tang et al. (2009) found the dominant arbuscular mycorrhizal fungi species in non-invaded sites were *Glomus mosseae* and *Glomus etunicatum*, while *Glomus geosperum* and *G. etunicatum* dominated *S. candensis* invaded sites. Besides, another reason for *S. canadensis* outcompete of native plant species was that its invasion reduced the AMF colonization of native species and then decrease its nutrient uptake (Zhang et al. 2007b; Jin et al. 2004; Sun and He 2010; Yang et al. 2014). Some studies showed that the root extracts of *S. canadensis* also affected the growth and activities of soil-borne pathogens *Pythium ultimum* and *Rhizoctonia solani* (Zhu 2011; Zhang et al. 2009b). The changed beneficial bacteria and pathogens in their introduced ranges affected the diversity of soil microbial community structure and function (Liao et al. 2013).

S. Canadensis also changed the soil physicochemical properties to affect its growth. The nutrient-related microorganisms changed in invaded habitats. The number of nitrite bacteria, aerobic azotobacter, sulphate reducer, ammonifier and aerobic cellulose-decomposer were increased, while the growth of denitrifier, anaerobic cellulose-decomposer and desulphate reducer were inhibited by this invasive alien weed. The soil properties (especially soil organic carbon, NH_4-N content, pH) significantly increased. Soil respiration and utilization of carbon sources were improved, and qCO_2 and functional diversity decreased (Zhang et al. 2009b; Liao et al. 2011).The results suggested that several soil chemical properties (e.g., organic carbon, NH_4-N, and pH) and microbial parameters (e.g., microbial biomass, basal respiration, substrate induced respiration, and functional diversity) might be used as indicators of *S. canadensis* invasive severity.

27.3.2 Ecological Adaptability

S. canadensis was able to form a community within 3 years after its colonization. In the process of invasions, *S. canadensis* initially forms numerous satellite populations, produces clones of individual plants with the aid of the underground rhizomes to fill gaps, and finally forms a single dominant species population supplanting the native species (Shen et al. 2004; Xu et al. 2006;Huang and Guo 2005). The rhizomes of *S. canadensis* are usually produced in late autumn and lie dormant during the winter months. Shoot extension occurs in the following spring (Patricia et al. 2011). Canada goldenrod flowers from July through September. Seeds are gradually dispersed during the autumn and winter.

S. canadensis can tolerate a fairly wide range of soil fertility and texture conditions, but is typically found in moist soils. Soil water content has greatly affected the leaf number and area. The biomass of the seedlings reached maximum in mesophytic habitats with 45% light intensity (Jiang 2008).

S. canadensis was tolerant to stress of heavy metals (Yang et al. 2007b). Xiang et al. (2010) found that the clean soil amended with Mn mining tailing was helpful to the growth of *S. canadensis*. *S. canadensis* also had higher tolerance to Pb than native plants, which enabled the invasive *S. canadensis* to outperform the native plant species and may have promoted its rapid invasion in Pb contaminated soil (Zhang et al. 2008).

27.3.3 Medicinal Effects and Other Uses

S. canadensis contain a wide range of bioactive compounds, such as flavonoids, polyacetylenes, terpenes, and saponins (Deng et al. 2015). Four new compounds, including two new clerodane-type diterpenes (solidagocanins A and B), 8-dehydroxymethylvisanol and 9-O-[3-O-acetyl-b-D-gluco- pyranosyl]- 4-hydroxycinnamic acid were isolated from this invasive plant species (Zhang et al. 2007a; Zeng et al. 2012; Huang et al. 2013a). The active ingredients are responsible for its antioxidant, antimicrobial, anti-inflammatory to spasmolytic and diuretic properties (Kang et al. 2010; Pietta 2000). The accumulation of certain polyphenols (such as flavonoids, tannis and phenolic acids) and their derivatives are mainly responsible for these potential health benefits (Deng et al. 2015). The antioxidant activities and contents of total phenolic and flavonoids varied with ripeness stages, tissue types and extraction methods (Deng et al. 2015; Qu et al. 2010). All leaf extracts exhibited antimicrobial activity against *Listeria monocytogenes* and *Staphylococcus aureus* (Deng et al. 2015). Flavonoids can effectively reduce cell peroxidation stress, prevent low density lipoprotein lipid peroxidation, exhibit some antimicrobial activities and the growth of algae, and prevent many diseases (Tijburgl et al. 1997; Qian et al. 2015). Petrol ether and ethyl acetate extracts were 50% toxic to four human tumor cell lines in the range of 15–50 μg/mL (Liu et al. 2007). The essential oils were screened for in-vitro cytotoxic activity against LOVO, HL-60, A-549, MDA-MB-435 and HepG2 human tumor cells, and they showed mild activities against A-549, MDA-MB-435 and HepG2 (Guo and Cui 2010; Huang et al. 2012; Fan et al. 2012).

S. canadensis can be used to prepare superabsorbent resin and high oil sorbents and its water or oil absorbency can reach 556.8 g/g and 21.036 g/g, respectively (Zeng and Yang 2012; Yang and Zeng 2012). The *S. canadensis* cellulose was modified using epichlorohydrin and trimethylamine to prepare an adsorbent for Cr (VI) and the adsorption quantity of Cr (VI) can reach 5.69 mg/g and the Cr (VI) removal rate is 91%. The combined pollution of Cu and Cd may confer a synergistic effect on the ecotoxicological effects of *S. canadensis* on the seed germination and growth of native plant species (Wang et al. 2015).

Fig. 27.3 The methods of controlling *S. canadensis*

27.4 Prevention and Control Methods

27.4.1 Manual Eradication

Manual methods can be used to eradicate it (Fig. 27.3). Plow and clear away the underground rhizome was the key to the complete eradication of *S. canadensis* (Qiu et al. 2012). Rotary tillage was often used to control this invasive plant species in the field, its control efficiency of plant height and fresh weight were about 50 and 70%, respectively. However, the density of *S. canadensis* was significantly increased after rotary tillage (Zhao et al. 2011).

27.4.2 Biological Control

Biological control was the ecological and safe ways to control this invasive plant species. Tang et al. (2013) found that the use of the combination of an indigenous fungal isolate of *Sclerotium rolfsii* SC64 and mechanical control was able to kill more than 90% of the ramets. Dodde (*Cuscuta japonica* Choisy) could restrain the growth and sexual reproduction of *S. canadensis*. The normal individuals of *S. canadensis* entered into the flower-bud stage in the middle of September, and the full flowering stage in the middle of October; in contrast, those parasitized by *C. japonica* produced flower-buds at the end of October, and its inflorescence length was only one-tenth of the controls (Jiang 2008; Sun and He 2010).

27.4.3 Chemical Control

Chemical control is one of the widely used ways to control *S. canadensis*. Several chemical pesticides can be used, such as fluroxypyr, metsulfuron-methyl and isopropyl glyphosate. Of them, fluroxypyr was the best choice (Qiu et al. 2012; Yang et al. 2012) (Fig. 27.3). Herbicidal effect is closely related to the age of plant population and spraying time of the pesticides. For example, March and April are the best periods to spay the pesticides to kill the twig and leaves, which is the most crucial to control the plant's growth (Zha et al. 2011). Generally, chemical control can achieve satisfied results when the plant to be controlled only in a small geographical area, but it is very expensive to be used in a large area.

27.5 Conclusions and Prospects

S. canadensis has caused great economic loss in China and had great potential distribution from 25° to 50°. Its allelopathic effects and control methods should be further carried out to discover its invasive mechanism and to realize its effective control. Besides, *S. canadensis* contain a wide range of bioactive compounds. How to research and develop the medicinal compounds to cure the disease should be further done.

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Chapter 28
Coastal Plain Yellowtops *Flaveria bidentis* (L.) Kuntze

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Abstract The coastal plain yellowtops, *Flaveria bidentis* (L.) Kuntze, a C_4 annual or perennial herb native to South America, has damaged local ecosystems and caused great economic loss in China. Its large seeds, strong ability to adapt to different environmental conditions and strong allelopathy to inhibit the growth of other plants lead to its expansion to Hebei, Henan and Shandong Provinces now. Based on biological and geographical characteristics, South, East and Central China are the potential distribution ranges of this weed. The main methods of controlling the invasive plant are chemical control, mechanical eradication and biological control. *Sorghum sudanense* (Piper) Stapf is the best alternative plant to control the growth of *F. bidentis*.

Keywords *Flaveria bidentis* • Alleopathy • Distribution • Ecological adaptability • Control methods

28.1 Introduction

The coastal plain yellowtops, *Flaveria bidentis* (L.) Kuntze (Asterales, Asteraceae) (Fig. 28.1), is a C_4 annual or perennial herb native to South America (Powell 1978). Now it is an successful invader worldwide (Gao et al. 2004). In addition to the capacity of producing a large number of seeds, *F. bidentis* has very strong ability to adapt to diverse environments and strong allelopathy to inhibit growth of other plants. It may heavily damage native ecosystems and cause serious economic loss (Peng et al. 2012).

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Fig. 28.1 *Flaveria bidentis* L. Kuntze

28.2 Spread and Damage of *F. bidentis* in China

In China, the introduction time and route as well as invasion history of *F. bidentis* in China remain unknown. The earliest record of its invasion can date back to 2001, when several plants were found on roadsides in the Nankai University campus, Tianjin, a city in northern China (Gao et al. 2004). Since then, *F. bidentis* has spread rapidly across northern China. It was also found in the Hengshui Lake of Hebei province in 2003 (Gao et al. 2004). In 2006, *F. bidentis* occurred in six counties of Tianjin, including Nankai, Hongqiao, Hangu, West Green, Jinghai and Ninghe. By the end of 2007, it has expanded to 89 counties in Hebei, five counties of Tianjin, and Anyang county of Henan province. In 2009, *F. bidentis* were found to have spread southeastwards, reaching Linqing and Dongchangfu, two counties of Shandong province (Ma et al. 2011; Bai et al. 2009). Human-mediated long-distance seed dispersal was speculated to have contributed to its rapid range expansion in northern China (Ma et al. 2011). The southern, eastern and central parts of China are the potential distribution areas of this weed (Ma et al. 2011) (Fig. 28.2).

F. bidentis have posed serious threats to crop growth and native biodiversity (Bai et al. 2009). It can dominate in a wide range of habitats, such as cornfields, cotton fields, orchards, lawns, roadsides, watersides and waste grounds, and can rapidly form almost monospecific stands within several years (Li et al. 2006). Several crops suffer serious damages from this plant, including wheat, corn, cotton and soybean. It was reported that yield loss of 32–95% were resulted in the cotton fields invaded by *F. bidentis* at densities 1–40 plants/m^2 (Wang et al. 2011; Peng et al. 2012).

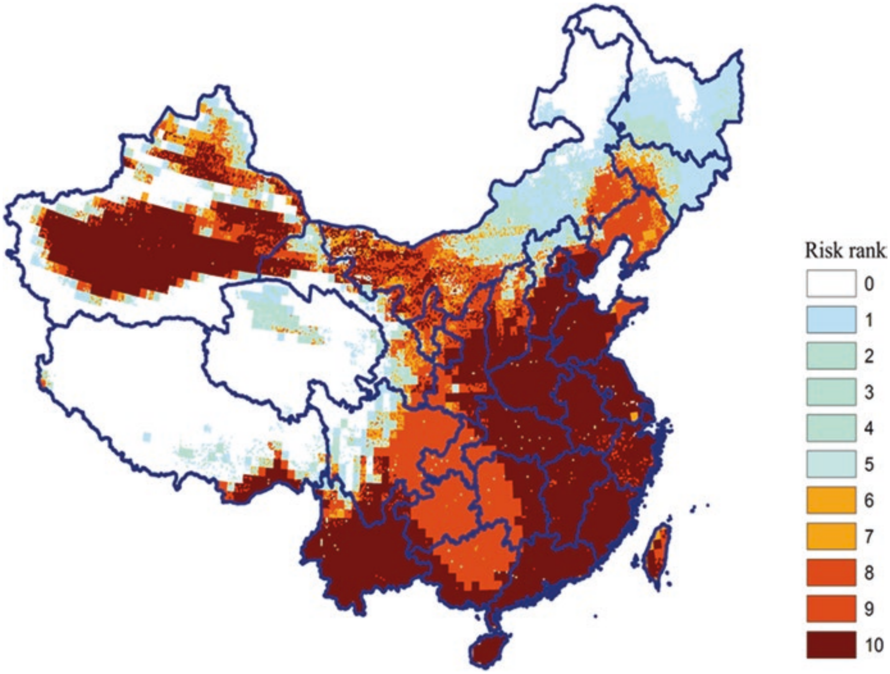

Fig. 28.2 Potential distribution of *F. bidentis* in China (Provided by Wang)

28.3 Biological Properties

F. bidentis has several important biological properties which contribute greatly to its invasiveness, such as high fecundity (Li et al. 2006; Powell 1978). In China, great research efforts have been made on the underlying mechanisms of invasion success.

28.3.1 Competitive Capacity

Rapid growth and low leaf construction cost contribute to its great competitive ability (Tu et al. 2013). The leaf construction cost is an important indicator in measuring the energy demand, and reflects the energy utilization strategy. It was found that *F. bidentis* has significantly lower leaf carbon and nitrogen concentrations than the co-occurring plants. Lower leaf construction cost has been hypothesized to give invasive plants a growth advantage through efficient energy utilization (Tu et al. 2013). However, *F. bidentis* was demonstrated to have a lower competitive ability when growing together with such native weed species that utilized same nutrient resources with *F. bidentis* (Lv et al. 2011).

28.3.2 Benefits from Changed Soil Microbial Community

Invasions of *F. bidentis* can lead to change of soil composition by diverting nutrients and minerals, which is beneficial to its growth (Li et al. 2011; Zhao et al. 2014;Zhao et al. 2015;Wang et al. 2016). One recent important finding was that *F. bidentis* could form symbiosis with arbuscular mycorrhizal fungi (AM fungi) (Li et al. 2015). *Glomus perpusillum* and *Rhizophagus intraradices* were gathered in the rhizosphere soil of *F. bidentis* during its invasions (Yin et al. 2015; Zhang et al. 2015). These AM fungi could improve the ability of *F. bidentis* to withdraw adverse conditions such as drought, through increasing soil water and mineral nutrient uptake, improving plant metabolic activities, and thereby promoting growth of *F. bidentis* (Chen et al. 2013). Moreover, the amount and kinds of phosphorus bacteria increased in the invaded soil (Ji et al. 2014). Interestingly, the changed soil microbial community may lead to changes of soil nutrient. For example, the content of soil organic matter, total nitrogen, and nitrate and ammonium nitrogen increased, while the phosphorus content decreased in its invaded habitats (Jia et al. 2015). Such changes in soil nutrients may facilitate further invasions (Zhang et al. 2010).

28.3.3 Allelopathic Effect

Allelopathic interference is one of the most important mechanisms for the successful establishment of invasive exotic weeds (Xu et al. 2007). *F. bidentis* releases allelochemicals to the environment mainly by litter fall and root exudates, as well as by eluviation (Feng et al. 2009). The allelochemicals released from roots are mainly octyl phthalate, phthalic-2-ethylhexyl ester, and butyl hydroxyl toluene (Feng et al. 2009). Phenolics were present in *F. bidentis* residues (Zhang et al. 2012b, c). *F. bidentis* had great allelopathic effects on some important crops, such as rice, wheat, maize, rape, cotton and mung bean (Peng et al. 2012; Xu et al. 2008; Zhang et al. 2008). For example, aqueous extracts of *F. bidentis* significantly reduced the germination percentage and root length of *Brassica pekinensis*, cabbage and rice (Ren et al. 2009; Zhang et al. 2008). The extracts (containing phenolics) from unburnt and burnt residues of the weed reduced the shoot length, root length and dry weight of wheat significantly (Zhang et al. 2012b). Some bioactive compounds from essential oil of *F. bidentis* were allelopathic to native plant species (Xie et al. 2010, 2012). Extraction solutions from the stem of *F. bidentis* showed a significant inhibition on the growth of crabgrass and red-root pigweed (Xu et al. 2008). The rhizospheric soils of *F. bidentis* weed plants have strong inhibitive effect on growth of the crops, such as corn, wheat, soybean and cabbage; seed germination rates and growth rates of the crop seedlings were significantly decreased. The morphology of affected crop seedlings was also significantly altered: color of seedling leaves became yellowish and the stems were much smaller compared with seedlings grown in normal soils (Xu et al. 2008).

28.3.4 Tolerance to Salt and Drought

F. bidentis has strong tolerance to salt and drought stress (Zhang et al. 2009). Its resistance to neutral saline soi1 was stronger than to alkaline saline soil (Chai et al. 2012). Seedling could tolerate 200-300 mmol/L of salt in the soils. This degree of salt-tolerance was higher than that of cotton (*Gossypium hirsutum* L.) and was equal to the halophyte *Suaeda helroptera* Kitag (Zhou 2010). Seeds could germination at the condition of 10% -15% of soil water content (Lu and Zhou 2006). Seedlings have a higher drought-tolerance ability than those of *Evonymus fortunei* and *Trachelospermum asiaticum* and is similar to that of *Festuca arundinacea* (Zhou 2010).

28.4 Managements

Several methods have been used in China to control *F. bidentis*, including manual control, biological control, chemical control and comprehensive utilization (Fig. 28.3).

Three pathogenic fungi, *Alternaria tenuissima*, *Colletotrichum* sp., and *Podosphaera xanthii*, have potential use in biological control of *F. Bidentis* (Sun et al. 2011). In some fields or habitats *F. bidentis* was found seriously infected by one or several of these pathogens and their growth and reproduction were suppressed. A pathogenicity test made in a greenhouse experiment showed that the three fungal pathogens were highly infectious and suppressive, with *P. xanthii* being the most pathogenic.

Replacement using alternative plants was a safe and effective method to control invasive plants (Gao et al. 2004; Zhang et al. 2011; Huangfu et al. 2010a, b). Some plants could suppress growth and reproduction of *F. bidentis* effectively, including *Sorghum bicolor* × *Sorghum sudanense*, *Sorghum sudanense, Astragalus adsurgens*, *Medicago sativa* and *Helianthus annuus* (Jiang et al. 2012, Zhang et al. 2012a; Yan et al. 2011; Ma et al. 2011; Chang et al. 2011). Of them, *S. sudanense* was thought to be the most effective one. When this plant are planted with *F. bidentis* at the ratio 1:3,

Fig. 28.3 Manual control (*left*) and chemical control (*right*) of *F. bidentis*

biomass and number of branches of *F. bidentis* significantly decreased. Having a stronger cold resistance than *F. bidentis*, *S. sudanense* germinates 10-day earlier. Moreover, *S. sudanense* grows fast and thus is able to cover ground rapidly, which can inhibit the photosynthesis of *F. bidentis* (Huangfu et al. 2010a; Ma et al. 2010; Jiang et al. 2012).

F. bidentis possesses a number of secondary metabolites that have application values, thus it can be hopefully controlled through comprehensive utilization (Xie et al. 2010, 2012). So far a great number of studied have conducted to identify and isolate bioactive compounds from essential oil of this plant (Wei et al., 2011a, b, 2012a, b; 2013, 2016;Yang et al. 2014; Dong et al. 2014). Extraction solutions from stems of *F. bidentis* were demonstrated to have herbicidal activity due to the existence of α-terthienyl (Xu et al. 2008; Huo et al. 2014).

28.5 Conclusions and Prospects

F. bidentis has caused great economic loss in China and will potentially distributed to southern, eastern, and central China. Lots of works have been carried out to try to discover its invasive mechanism. But there are still many problems to be solved, such as the role of microbe in its invasion, choice of appropriate plants for replacement, and the effective methods of monitoring and preventing its further spread.

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Chapter 29
Alligator Weed *Alternanthera philoxeroides* (Mart.) Griseb.

Mengzhu Shi and Jianwei Fu

Abstract *Alternanthera philoxeroides* is one of the worst weeds in China. The successful invasion of alligator weeds is not only associated with its specific biological characteristics, such as vegetative propagation, high phenotypic plasticity, extensive adaptability, strong stress resistance and so on, but also related to the invasibility of the habitat it invaded, lack of natural enemies and global climate change. It is essential to integrate physical, chemical and biological means, intensify regional management and adopt time-and site-specific optimal measures for the control of alligator weed. And more attention should be paid to the research on its phenotypic plasticity, adaption of bio-control agents and the use of *A. philoxeroides*.

Keywords *Alternanthera philoxeroides* • Damage • Invasion mechanism • Control strategy • Utilization

29.1 Introduction

The alligator weed, *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae), native to South America (Brazil, Uruguay and Argentina), is a vicious weed worldwide. It is listed as one of the 16 most serious alien invasive species of the world (MEPPRC and CAS 2003). In China, it is usually named as Shui-hua-sheng. When growing on land, *A. philoxeroides* tends to displace other plants such as crops or native vegetation; when growing in fresh water, it can conquer the entire water surface, thereby reducing oxygen exchange and affecting aquatic plants and animals (Coulson 1977).

During the War against Japan, alligator weed was introduced into Shanghai (in 1930s) by Japanese as a forage plant. At the end of 1950s, alligator weed was popularized for cultivation as pig feed. Since then it grew and multiplied rapidly in

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southern China. Nowadays, the weed has been widely distributed in 18 provinces, especially in Fujian, Guangdong, Hunan, and Hainan provinces. In the downstream of Yangtze River, alligator weed has spread widely and caused serious damages (Lin et al. 1990; Zhang et al. 1993; Tan 1993).

29.2 Effects on Ecosystem in China

A. philoxeroides can invade both land and water bodies. Due to the high invasive capacity, it often causes enormous economic loss and exerts negative impacts on local ecosystems.

First, in crop fields and orchards, the underground stems of alligator weed can spread extensively forming the root and stem systems like cobwebs. Meanwhile, the above-ground parts grow rapidly to form dense foliage. As a consequence, the weed competes with crops for fertilizer and water, which leads to loss of crop yields (Yu et al. 2008; Lin et al. 2005). In China, the resulted yield losses can be as high as 45% in rice, 36% in wheat, 19% in maize and 5–15% in vegetables (Yin 1992; Tan 1994).

Second, the thick pad-like substances formed by the weed may stop water flow, block channels, obstruct water traffic and transportation, and hamper flood-releasing and water-log draining (Coulson 1977; Wang and Wang 1988). For example, at the ditches and wetlands of Dongting Lake areas in Hunan province, China, the alligator weed reached a coverage 70.5 and 88.4%, respectively, in 2006, which severely blocked the rivers, affected aquaculture, reduced species richness, causing serious damage to the local agricultural eco-economic system (Zhou et al. 2007). In Chongqi, water quality was ever seriously reduced by rotted alligator weed, as a result, fish in pond died and over 200 ha of fishponds were abandoned (Zhang et al. 1993).

Moreover, *A. philoxeroides* may reduce the species diversity of invaded ecosystem. By posing allelopathic effects, *A. philoxeroides* can perform interference competition, exclude and strangle native species. For this reason, the weed can occupy ecological niche quickly. In China, the weed is able to inhibit growth of a number of native plant species (Lin et al. 2005). The aqueous extracts from *A. philoxeroides* could affect germination and growth of *Vicia faba*, *Zea mays*, *Raphanus sativas* and *Lactuca sativa* seedlings (Xiong et al. 2011; Song and Zhong 2013). Overall, invasion of *A. philoxeroides* can decrease the stability and species richness of native community, and can even replace some native species. It was reported that, in the seriously invaded vegetation community, 45% of native species disappeared (Guo et al. 2009).

Alligator weed also invades park, woodland, lawns, lakes and other scenic spots, which subsequently affects tourism and damage forestation (Lou et al. 2002). Some solid or garbage difficult to decompose in the water tend to congregate around the stems and roots of alligator weed. This may provide a good place for mosquito oviposition, thereby supporting a large number of mosquitoes and bringing harms to human health (Barreto et al. 2002).

29.3 Invasion Mechanism

In the past decades, researches have been extensively conducted on invasion mechanisms of *A. philoxeroides*. A number of factors were found to greatly contribute to its rapid spreading in China, including strong reproductive capacity, existence of various ecotypes and biological types, strong competitive ability, high resistance to barrenness and pollution, and absence of competitive organisms or natural enemies (Ma and Wang 2005).

29.3.1 Reproductive Capacity

Alligator weed is a well adapted species capable of reproducing both sexually and asexually. Throughout its introduced range, *A. philoxeroides* rarely produces viable seeds and reproduces mainly by vegetative propagation via storage roots and stems. It was showed that a segment of stolon and rhizome with only one node could survive under suitable conditions (Lin and Qiang 2004). Because of this, if the rhizomes and stolons are brought to the ground or to other places through ploughing, *A. philoxeroides* will easily get established and build up a large population in a short period of time (Lin et al. 1990; Wang and Wang 1988; Zhang et al. 1993). The experiment under three different niches showed that the aerial part of alligator weed had a strong spreading ability (Lin and Qiang 2004), which is conducive to the invasive and spread of the weed.

29.3.2 Phenotypic Plasticity

A. philoxeroides lives in both terrestrial and aquatic habitats. To acquire adaptation to various habitats, the weed had undergone some morphological and anatomical changes (Huai et al. 2003; Ma et al. 2003). For example, to adapt land conditions, it thickens epicuticular wax, increases the layers of collenchyma cell and the vascular bundles of bast fibre, and arrange palisade tissue cells closely (Tao and Jiang 2004; Zhang et al. 2001). Such adaptive changes have been extensively observed in various geographic populations of China, suggesting that phenotypic plasticity has significantly contributed to the invasion of this weed (Pan et al. 2006). In addition, *A. philoxeroides* have also evolved a plastic response to shading (Zhang et al. 2015) and temperature (Chu et al. 2014) in its introduced range. So far in China phenotypic plasticity is considered as one of the most important reasons for *A. philoxeroides* adaptation to diverse environments.

29.3.3 Tolerance to Adverse Environments

Alligator weed has strong resistance to rising temperatures and drought, and it is not sensitive to low temperature. Its roots and stems can still live after freezing 3 ~ 4 days in minus 5–3 degrees centigrade (Lin et al. 1990; Zhang et al. 1993). The survival rate of *A. philoxeroides* remained 90% after a 120-d submergence (Wang et al. 2008b). Alligator weed also has strong tolerance to salt, acid, alkali, barrenness and pollution (Liu et al. 2013; Guo et al. 2012b; Song et al. 2011; Xu et al. 2010). So it can grow and reproduce successfully under a variety of adverse environments.

29.4 Management

29.4.1 Mechanical/Manual Control

Mechanical and manual techniques are good options for the control of *A. philoxeroides* when its infestation is limited in a small area, where all above- and below-ground materials can be removed. But the mechanical control method has some limitations. For example, after the above-ground stems of *A. philoxeroides* are cut, new stems maybe grow quickly because of the strong reproductivity (Chan and Chen 2010). For this reason, mechanical control and plough should not be used to control terrestrial alligator weed, otherwise the weed would be cut into more stems and large number of new plants would be produced from the stems. To increase control efficiency, roots of alligator weed should be dug out, collected and dried in the sun and burn down. The aquatic alligator weed should be salvaged underwater, then buried deeply or used for producing fertilizer (Zhang 1994). As mechanical and manual techniques can be used only in a small scale, the process must be repeated, and the cost is high, their application is much limited.

29.4.2 Biological Control

Biological control is characterized by bringing little pollution to environment, effectively protecting natural enemies, helping in achieving sustainable control, and rarely causing pesticide-resistance in targeted pests. So far several biological control agents of alligator weed have been introduced to China, or have been identified in local native community. They include insect natural enemies, parasitic fungi and allelopathic chemicals.

Agasicles hygrophila Selman and Vogt, a flea beetle native to Argentina, was introduced to China in 1980s by Chinese Academy of Agricultural Sciences (Fig. 29.1). Its life history characteristics (Wang et al. 2008a; Chen et al. 2009;

Fig. 29.1 *Agasicles hygrophila*, natural enemy of alligator weed

Table 29.1 Control efficiency of *A. hygrophila* on *A. philoxeroides*

Site	Time	Area	Number of flea beetle released	Control efficient (percentage of damage)
Changsha City, Hunan Province	1988	3000 m^2	800	100%
Chongqing City, Sichuan Province	1990	4.87 hm^2	2000	100% (45 d)
Changshou County, Sichuan Province	1991	140,000 m^2	200	100%
Nanning city, Guangxi Province	1991	200,000 m^2	150	100% (30 d)
Yueyang City, Hunan Province		667 hm^2	16,000	90%
Shanghai City		320,000 hm^2		100%

Guo et al. 2014), adaptability (Ma 2009; Zhao 2009; Xie 2012; Guo et al. 2012a; Li et al. 2013; Fu et al. 2012), rearing techniques (Liu et al. 2009) and control efficacy (Huang 2010; Xie 2012; Shi et al. 2012) have been extensively investigated in this country. *A. hygrophila* was reported to have been released in Sichuan, Hunan, Guangxi and Yunnan provinces and got established in Chongqing and Changsha (Lin et al. 1988; Huang et al. 1996; Li et al. 2000) (Table 29.1). The flea beetle effectively reduced the large floating mats of alligator weed and prevented their reformation (Fig. 29.2). In early seasons, alligator weed normally extends up to several meters over water when the flea-beetle population has not increased, but afterwards the weed is restricted to the bank as the beetle density increases (Li et al. 2000; Wu et al. 2000; Liu et al. 2010). Pupation rate and control efficacy of the beetle are affected primarily by the thickness of vascular cylinder, pith cavity parenchyma and the density of cortex parenchyma of its host plant (Ma and Wang 2004). Ecotype of alligator weed was found to be the key factor affecting the pupation of *A. hygrophila*. *A. hygrophila* pupate more well on the aquatic type than terrestrial type of alligator weed, because the vascular cylinder cells and parenchyma cells of

Fig. 29.2 Comparison of control alligator weed before and after releasing *A. hygrophila* in Changsha, Hunan province

the terrestrial weed are both thickening, which bring difficulties and obstacles for *A. hygrophila* to pupate (Ma et al. 2003). Temperature can also affect *A. hygrophila* population (Fu et al. 2011; Li et al. 2012). Based on the observation of *A. hygrophila* adult reproduction, leaf consumption, egg hatching rate, larval and pupal survival and development in response to low non-freezing temperatures, it was predicted that *A. hygrophila* will extend further northwards under global warming. Apparently, the increased temperature will allow the beetle to overwinter more well, which will make it an effective biological control agent for alligator weed in northern regions of China (Guo et al. 2012a). But high temperature has adverse impacts on fecundity of *A. hygrophila* (Zhao 2009).

Bio-control of alligator weeds with microbial pathogens has been studied extensively and some achievements have been made since the 1960s. Two fungal species of potential applicability have been found in Chongqing, China: a *Colletotrichum* sp. and a *Fusarium* sp. (Tan 1993). Greenhouse and field trials revealed that all alligator weed were diseased and then killed after inoculated with *Fusarium* sp. at concentrations 1.0×10^5 and 1.0×10^6 spores·ml^{-1}, with the control efficiency similar to the one resulted from glyphosate treatment (Tan et al. 2002). *Nimbya alternantherae* is another highly phytopathogenic fungus of *A. philoxeroides*. After it was used twice with the suspension 8×10^4 spore·ml^{-1}, the weight of weed leaf decreased by 92.9% in 10 days (Xiang et al. 2002). When the weed are attacked by *N. alternantherae*, the equilibrium between production and scavenging of reactive oxygen species (ROS) can be disturbed which leads to a sudden increase in intracellular levels of ROS. Such a change can significantly damage leaves of *A. philoxeroides* (Nie et al. 2013; Li 2011).

Allelopathy helps to restrain the growth of *A. philoxeroides*. For example, *Artemisia annua* L., one plant having a strong allelopathic effect against shoot and root of *Brassica napus* (Tan et al. 2011), is capable of inhibiting *A. philoxeroides* (Chen et al. 2008). The underlying mechanism of such an inhibiting is to be investigated.

29.4.3 Chemical Control

Some herbicides have been tested for their control efficacy against the weed. Glyphosate, fluroxypyr and some compound herbicides were demonstrated to have good effects (Zhu et al. 2002; Zheng 2005; Shang and Chen 1997; Yao et al. 1997). Glyphosate had excellent control to aboveground parts of *A. philoxeroides*, but had no demonstrable effect on the underground stems (Li and Hu 2008). Fluroxypyr could be used in both aquatic and dry land areas, whereas glyphosate was more suitable for dry land use. To reduce side effects of chemicals on environments, new herbicide, such so the botanical ones, are to be developed.

29.4.4 Utilization

At present, the utilization of *A. philoxeroides* is mainly embodied in the fields of agriculture, pollution control and medicine. Due to the high protein content, it can be used as animal husbandry and fishery feed (Li et al. 2013). Breeding fish with *A. philoxeroides* not only saves the cost, but also has antibacterial, antiviral effect to prevent fish diseases (Wang and Zhao 2010). Feeding alligator weed can also increase the amount of cow lactation and the weight of cattle (Wang and Chen 1986). *A. philoxeroides* can be used to control pollution, because it possesses the capacity to enrich 3-metalcations of Mn^{2+},Pb^{2+}, Cd^{2+}, which were mainly accumulated in the shoots (Song and Zhong 2013). Pharmacological researches showed that the extraction of *A. philoxeroides* has antiviral effects (Fang et al. 2007; Li et al. 2013; Ding et al. 2011). Moreover, *A. philoxeroides* has great potential to be a botanical pesticide. The artificial community with *A. philoxeroides* has obvious molluscicidal effect (Wu et al. 2010), and the *A. philoxeroides* saponin was demonstrated to have some toxicity aginst *Pomacea canaliculata* (Wang et al. 2011).

29.5 Conclusions and Perspectives

A. philoxeroides as an invasive plant, its damage, invasion mechanism, and integrated management has drawn more attention. *A. philoxeroides* has undertaken phenotypic plasticity as a major strategy to adapt to the new environments in the introduced ranges, and this aspect was a research hotspot in the past decade. Although physical control and chemical control has the advantages of quick effect, biological control is arising an environment-friendly, sustainable control method for this weed. The flea beetle *A. hygrophila* is the most effective predators of *A. philoxeroides*, but its control efficacy may be limited in cold regions and seasons. How this beetle overwinter need to be further studied. The fungal biocontrol agents was poorly studied, but they have a high use potential and should be paid more attention in the future.

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Chapter 30
Water Hyacinth *Eichhornia crassipes* (Mart.) Solms

Jianwei Fu, Mengzhu Shi, and Jianyu Li

Abstract Water hyacinth, *Eichhornia crassipes*, is considered as one of the world's worst weeds. They successfully invaded lakes, ponds, canals, and rivers. It is still rapidly spreading into many countries,influencing local environmental and agriculture cultural problems. The aim of this chapter is to summarize the effects caused by *E. crassipes* invasions in china, discuss the causes and consequences of the effects, and propose control creative *E. crassipes* methods. At present, solutions for preventing the spread of water hyacinth mainly include three general categories: physical, chemical and biological control. Water hyacinth population can be reduced and controlled by using an integrated management approach, and promote a long term sustainable approach for effective water management.

Keywords *Eichhornia crassipes* • Ecosystem Effects • Invasion Mechanism • Control Strategy

30.1 Introduction

Water hyacinth *Eichhornia crassipes* (Mart.) Solms (Pontederiacea) is an aquatic free-floating plant. It is native to South America and now has been widespread, ranked as one of the top ten highly invasive weeds in the world (Gao and Li 2004; Wang 2003). In China, water hyacinth was first introduced into Taiwan in 1901 as an ornamental plant and into the mainland during 1930s (Diao 1983). During early years, this plant was widely grown as an animal fodder in southern China, and then it escaped and became naturalized. By the end of twentieth century, it has been widely present in 19 provinces in northern, eastern, central and southern parts of China. This weed has caused serious ecological damages in several regions, in particular in the Yangtze River Basin and southern China (Ding 1995a; Wang 2003).

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30.2 Damages

Under suitable environments, water hyacinth can proliferate very rapidly and form a large population within short time. It causes serious ecological, economic and social problems in invaded regions.

30.2.1 Effects on Aquatic Ecosystem

Water hyacinth seriously affects aquatic organisms and biodiversity. Large-scale covering of water hyacinth on water surface blocks sunlight, reduces photosynthesis, and threatens local communities, thereby reducing native species diversity (Huang et al. 2007; Schlettwein and Bethune 1992; Wu et al. 2004). In particular, this weed can decrease the concentration of dissolved *oxygen* in *wate*r and suffocate some aquatic animals (Li et al. 2004). It was reported that in the freshwater bodies abundant with water hyacinth, the dissolved oxygen was only 0.6 $mg \cdot L^{-1}$, which was below the threshold (3 $mg \cdot L^{-1}$) required for fish survival (Zheng 2006). In Dianchi Lake of Yunnan province, the high-class plant species decreased from sixteen in 1960's to three only in 1990's , and fish species decreased from fifteen to five (Hong 2004; Wu 1993).

Another serious ecological problem caused by water hyacinth invasions is the changes in structure and function of local aquatic ecosystem. This plant is highly efficient at taking up calcium, magnesium, sulfur, manganese, aluminum, copper, molybdenum zinc, nitrogen, phosphorus and potassium (Cai et al. 2002; Tan et al. 2009). As a result, the circulation of these chemical elements, a proportion of which are closely with native species' growth and development, can be changed tremendously. In addition, as water hyacinth dies, it sinks, decomposes and releases large amount of nutrients, resulting into serious eutrophication (Zheng 2006).

30.2.2 Effects on Economics and Human Life

Large and dense monocultures of water hyacinth covers water surface of lakes and rivers (Fig. 30.1), which blocks waterways (Fig. 30.2) and impacts tourism activities, hydroelectric power and irrigation. The formed dense mats can seriously influence aquatic production, such as reducing fish catches. In 2009, for example, water hyacinth caused the death of 349 tons of fish and a huge economic loss of over 780,000 USD in Xiongjiang Town, Minhou County, Fujian province (Xiao et al. 2009). The direct economic loss resulted from water hyacinth damage was estimated to be 1.2–1.5 billion USD each year in China (Table 30.1) (Wang 2003). In recent years, direct economic loss and the control cost associated with water hyacinth have increased greatly.

Fig. 30.1 Thick mat at Dazhangxi River in Minhou county, Fujian province

Fig. 30.2 Block waterways and clog waterway transport

Table 30.1 Damaged and economic loss caused by water hyacinth

Region	Year	Damaged area	Salvage cost
Wenzhou, Zhejiang Province	1999		1,500,000 USD
Putian, Fujian Province	1999		750,000 USD
Nanping, Fujian Province	–	800 hm^2	–
Dianchi,Yunnan Province	–	1000 hm^2	–
Taiwan Province	–	61,000 hm^2	3,200,000 USD
Shanghai	2002		12,000,000 USD

Water hyacinth also impacts human health. This is because deceased or rotten water hyacinth can deteriorate water quality. They also provide a good condition for oviposition and breeding of mosquitoes (Lei et al. 2008).

30.3 Invasion Mechanism

There are several reasons for the overspreading of water hyacinth in China, including high reproductive capacity, great adaptability to diverse aquatic conditions and lack of natural enemies.

30.3.1 High Reproductive Capacity

Water hyacinth has a great capacity in both sexual and asexual reproduction. By self- pollinating, the plants can produce a large number of seeds (> 3000 seeds per inflorescence) in the long life history (Barrett 1980; Diao 1983; Perkins 1976). The small, long-lived seeds sink and can remain viable in sediments for 15–20 years (Matthews 1967). Sexual propagation is the main reproduction mode of water hyacinth. The single apical meristem on each stem tip can be vegetative, producing leaves with axillary buds, and the axillary bud below the inflorescence differentiates into a continuation shoot. Through this process, a new apical meristem may be produced allowing leaf production to proceed. If the axillary bud does not form a continuation shoot, then it produces a stolon. A new plant can be produced by the apical meristem in 5 days under suitable environmental conditions (Wu et al. 2001).

30.3.2 Wide Ecological Adaptability

Water hyacinth can adapt to a diversity of habitats. It can grow in water and mud, and has no strict requirements for weather conditions. Water hyacinth begins to grow as ambient temperature rises to 13 °C, and it can grow very well at 25–35 °C

(Gao and Li 2004; Wu et al. 2001). This is the major reason why this plant distributes so widely between 40°N and 40°S latitude (Ding et al. 2001). Water hyacinth is also tolerant to a wide range of water pH and nutrient level, so it can grow in various types of aquatic environments (Gao and Li 2004; Howard and Harley 1998).

Another most important factor promoting the spread of water hyacinth in China is water eutrophication. In recent years, a large number of wastewater and solid waste from industry and agriculture were flowed into rivers, which causes the water eutrophication and provides the suitable nutritional requirement, such as nitrogen, phosphorus and other organic materials, for the growth and spread of water hyacinth (Chen et al. 2013; Hong 2004). Moreover, water hyacinth could result into an increase in biochemical oxygen demand (BOD) and nutrient loading in water following plants' death, corruption and decomposition, which in turn accelerates the water eutrophication (Wu et al. 2004). An example comes from Huizhou City, Guangdong Province. One third area of the surface of Tonghu Lake was ever covered by water hyacinth, which heavily blocked water current. It was shown that water eutrophication caused by industrial wastewater and sanitary waste was the most important reason for such a damage (Ji et al. 2005).

30.3.3 Lack of Natural Enemy

Lack of natural enemy often was considered to have contributed to the water hyacinth's wide spread (Xu 2007). In Uruguay, Trinidad, Argentina, the United States, or other countries of South America and India, there are more than 70 kinds of arthropods that feed on water hyacinth (Chen 1996). In china, however, there are rarely effective native natural enemies are present to prevent water hyacinth.

30.4 Control Strategy

The effective management strategy against water hyacinth includes the regional control and comprehensive utilization. The control methods included physical control, using chemical herbicides, and releasing biological control agents.

30.4.1 Physical Control

In China, the artificial mechanical salvage is the main method controlling water hyacinth. As it is friendly to environments and aquatic ecosystem, massive salvage operations have been implemented in Shanghai, Zhejiang and Fujian Provinces (Chen and Ling 2004; Wu et al. 2003)(Fig. 30.3). Specific salvage ships and croppers have been designed and manufactured for this purpose (Xu 2008) (Table 30.2).

Fig. 30.3 Artificial mechanical salvage water hyacinth

Table 30.2 Machinery control equipment of water hyacinth

Model	Company	Capacity (8 h)
WH1800	Shaoxing Farm Machinery Management Station	120 t
ZS-800	Zhejiang Shipyard Hydraulic Dredging Engineering Co., LTD	3–8 hm^2
P-150	Foshan Institute of Agricultural Machinery	0.8–1.6 hm^2
Small mower in river	Complete set of Modern Agricultural Equipment co., LTD of Shanghai Electric Group	0.8–1.2 hm^2
SGJ20	Jiangsu Water Conservancy Machinery Manufacturing Factory	4.8–6.4 hm^2

However, this control method is expensive due to the high cost to labors. For example, 165,000 USD for artificial salvage of water hyacinth was cost in Xiamen, Fujian Province in 2007 (Chu 2010). Each year, at least 15 million USD was spent into salvage and control water hyacinth in China (Wang 2003).

30.4.2 *Chemical Control*

Herbicides gramoxone (or paraqua), glyphosate, bensulfuron methyl are often sprayed to control water hyacinth in China (Chen et al. 2004, 2005; Ding et al. 1998a; Pan and Wang 2004) (Figs. 30.4 and 30.5). Although water hyacinth can be controlled rapidly (usually in 1 week), this method has some shortages. The most evident one is that some herbicides may pollute water and harm aquatic organisms. Thus, cautions should be taken when herbicides are sprayed in lake basins and the areas near human habitations (Ding et al. 1998b; Wu et al. 2003).

Fig. 30.4 Comparison of control water hyacinth before and after spraying herbicides at Liulang river in Minhou County, Fujian Province

Fig. 30.5 Comparison of control water hyacinth before and after spraying herbicides in Minqing County, Fujian Province

30.4.3 Biological Control

Biological control is considered as the best sustainable and effective method to control water hyacinth, because it is normally effective, low cost, and friendly to environments. A number of insect natural enemies, pathogens and allelopaths have been available (Table 30.3). The weevils *Neochetina eichhorniae* and *Neochetina bruchi* are the most successful enemies against water hyacinth (Chen 1996) (Fig. 30.6). Both weevils were introduced from the United States and Argentina to China by Chinese Academy of Agricultural Sciences in 1995 (Ding 1995a, b). They are highly host specific in China, and thus can be used safely for water hyacinth control (Chen et al. 2005; Ding et al. 2002).

The weevils have been released in some regions of China to control water hyacinth. For example, In August, 1996, 1000 *N. eichhorniae* adults were released in an area of 1372 m^2 in Wenzhou, Zhejiang province, after 3 years the control efficiency reached over 90% (Ding et al. 2001). The deficiency of using weevils is that they work much slowly, as compare with physical control or use of herbicides, but the

Table 30.3 Biological control agents of water hyacinth, *E. crassipes*

Natural enemy	Class or order	Family	Species
Insect	Coleoptera	Curculionidae	*Neochetina bruchi*
			N. eichhorniae
	Lepidoptera	Noctuidae	*Bellura densa*
		Pyralidae	*Haimbachia infusella*
			Sameodes albiguttalis
	Orthoptera	Acrididae	*Cornops aquaticum*
	Galumnidae		*Orthogalumna terebrantis*
Fungi	Hyphomycetes	Cercospora	*Cercospora rodmanii*
			Cercospora piaropi
		Dematiaceae	*Alternaria eichhorniae*

Fig. 30.6 *Neochetina bruchi* (*left*) and *N. eichhorniae* (*right*) adults. (Photo courtesy of W. C. Durden, USDA, ARS.)

control effect will persist a long time after the released weevils have established populations in the wild (Ding et al. 2001).

In addition to natural enemies, pathogens can also be used as biocontrol agents of water hyacinth (Table 30.3). Pathogens *Cercospora piaropi* and *Alternaria eichhorniae* are considered to be potential mycoherbicide (Ding et al. 2006; Wang and Chu 2006a, b) Two isolate of *C. piaropi*, WH9BR and BA-57, introduced from University of Florida, were studied to determine the optimum light, temperature, pH, carbon and nitrogen sources, moisture and medium for its mycelial growth, sporulation and spore germination (Wang and Chu 2006a, b). A pathogenic strain *Alternaria* sp.WH56 was isolated from naturally diseased water hyacinth collected from Zhejiang province, and its biological characteristics were studied. After sprayed with *Alternaria* sp.WH56, plants reached morbidity 100% and the disease index 46.47% after 1 week, and the disease index increased to 100% after 1 month (Zhao 2008).

Botanical extracts could also be used to prevent growth of water hyacinth. Fox example, lantana leaf extract was ever used to prevent the water hyacinth by foliar spraying. Necrosis spots were detected after 24 h and 52% leaves were damaged after 5 days. Then after 13 days, almost all leaves of the plants fell into decay. The average number of leaf buds in the treated plants decreased significantly 11 days after sprayed (Zhang et al. 2005; Zheng et al. 2006).

30.4.4 Integrated Management

Overall, it is not sustainable to separately apply the physical, chemical, or biological methods to control water hyacinth in the field. Therefore, an integrated management strategy is to be adopted, by comprehensively using these methods (Ding et al. 1999; Zheng 2010).

First of all, the population monitoring and prediction of water hyacinth is important and to be conducted extensively. Second, mechanical salvage should be implemented during the seedling stage of water hyacinth, in particular in the middle and upper parts of rivers. In outbreak areas, it is necessary to apply high efficiency, low toxicity and residue chemical herbicides to control the water hyacinth.

Thirdly, releasing natural enemies, combined with the use of low toxicity herbicides, will achieve a long-term control effect over the large areas with water hyacinth outbreaks. Feeding of released weevils make wounds on water hyacinth, through which more herbicides would enter water hyacinth. Laboratory experiments suggested that releasing two weevils per plant together with the use of 200 $mg \cdot L^{-1}$ glyphosate was efficient to control the weed, where 100% of water hyacinth died at 15 day after glyphosate application (Zheng 2010).

30.5 Conclusions and Perspectives

E. crassipes was considered one of the worst weeds because it has caused serious ecological and social problems in the world. In China, much research work has been given to the biology and management of *E. crassipes*. Yet, efforts are still needed to uncover the mechanisms behind the invasion and spread of this weed in China, which would be important for developing prevention and control methods. In future, the fields to be focused include population ecology of the weed in various habitats, genetic variation and evolution, and the interaction between the weed and natural enemies.

Biological control has been long suggested as the best means for controlling water hyacinth in China, and now it is still concerned by many researchers. However, further work is needed to improve the current biocontrol methods, by considering more carefully the relationship between weevil behaviors and the herbicides use and mechanical/physical control operation. Besides the weevils, other kinds of control agents are to be developed.

Water hyacinth has been considered to be recycled in the real life. In fact, water hyacinth has a very strong purifying function to water, especially to eutrophic water, and has been confirmed as the best plant to remove nitrogen and phosphorus (Li et al. 2011; Zheng et al. 2011). In recent years, it has been widely used in the research and practice of sewage, industrial wastewater, eutrophication lakes, rivers and so on. However, further studies should be carried out with respect to the purification mechanism and its nutrient release mechanism. It might be hoped to develop a real method of utilizing water hyacinth as a resource.

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Chapter 31
Saltmarsh Cordgrass *Spartina alterniflora* Loisel

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Abstract Saltmarsh cordgrass *Spartina alterniflora* Loisel, native to North America, is now recognized as one of the most harmful invasive plants in China. Since 1990s, *S. alterniflora* has successfully invaded the Yangtze River estuary, a globally important eco-region. The invasion has resulted from the plant's abilities to adapt to the estuarine environments and human disturbances. *S. alterniflora* invasion has multiple ecological consequences to the estuary. The invader out competes native plants such as *Scirpus mariqueter* and *Phragmites australis*, and even causes local extinction of *S. mariqueter*. In addition to changing native soil microbial community diversity and structure, *S. alterniflora* may carry a fungal pathogen (*Fusarium palustre*) from its native range which seriously damages native *P. australis*. *S. alterniflora* also significantly alters community structure of soil nematodes and macrobenthonic invertebrates, and changes arthropod abundance. Moreover, the abundance of shorebirds is threatened by *S. alterniflora*-induced changes in food resources and physical habitats. For ecosystem functioning, *S. alterniflora* invasion increases primary productivity and alters carbon and nitrogen cycling. In the face of challenges, controlling *S. alterniflora* has been successfully implemented in the Chongming Dongtan of the Yangtze River estuary, but further studies are needed to find an efficient way to control the invasion at a large scale.

Keywords *Spartina alterniflora* • Biodiversity • Invasion mechanism • Ecosystem functioning • Ecological restoration • Yangtze River estuary

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31.1 Introduction

The saltmarsh cordgrass *Spartina alterniflora* Loisel, native to the Atlantic and Gulf coasts of North America, is a perennial rhizomatous C_4 grass in the family Poaceace. As an ecosystem engineer, it was intentionally introduced into China in 1979 for erosion control, soil amelioration, and dike protection in coastal mudflats (An et al. 2007). However, this exotic plant spreads rapidly along the eastern coast of China, where by the year 2000 it had covered more than 112,000 ha from Dandong in Liaoning Province to Beihai in Guangxi Zhuang Autonomous Region (An et al. 2007). Because of its biological traits such as rapid growth, high productivity, high tolerance to salt, and well-developed belowground system (Crooks 2002), *S. alterniflora* replaces native plants, threatens local ecosystems, and causes considerable losses of regional economies. Considering these negative impacts, the State Environmental Protection Administration (SEPA) of China has listed *S. alterniflora* as one of the most harmful invasive species (Wang 2011).

The Yangtze River estuary, a globally important ecoregion covering a large portion of Shanghai and fractions of Jiangsu and Zhejiang Provinces, has diverse coastal wetland ecosystems (e.g., shallow open waters, mudflats, salt marshes, and brackish ecosystems). The estuary offers important habitats for migratory birds on the East Asian-Australasian Flyway (Ma et al. 2004). The estuary, however, is

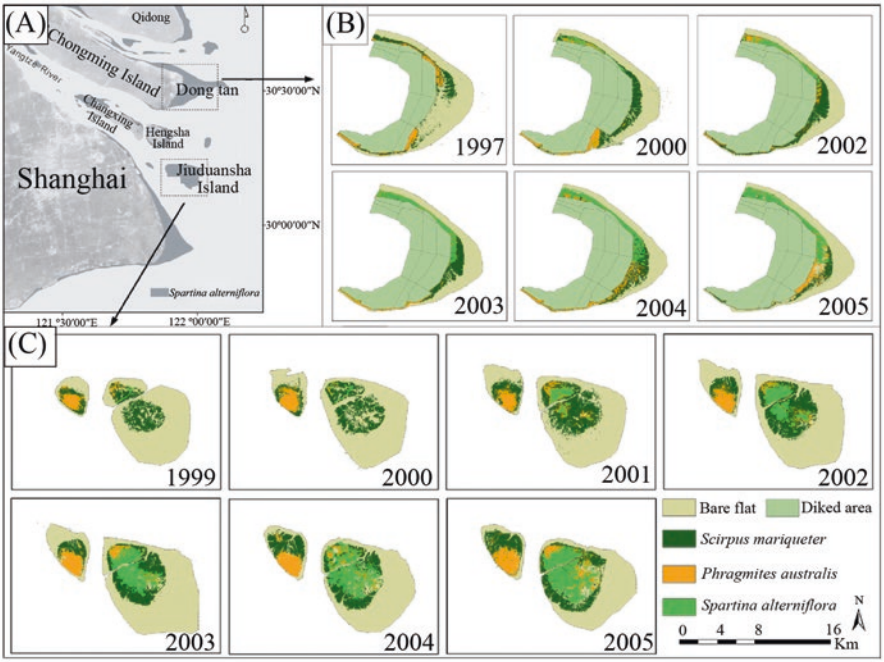

Fig. 31.1 Distribution of *Spartina alterniflora* (**a**) and its expansion in the Chongming Dongtan (**b**) and Jiuduansha (**c**) of the Yangtze River estuary, China (From Li et al. 2009)

threatened by invasive *S. alterniflora*, particularly in the Chongming Dongtan wetland where it was first found in 1995. After that, *S. alterniflora* was intentionally introduced to the Jiuduansha Islands in 1997 and to the Chongming Dongtan wetland in 2001, leading to its rapid expansion in the Yangtze River estuary (Fig. 31.1) (Li et al. 2009). By 2005, this plant formed monocultures that occupied 37.1% and 49.4% of the vegetated areas in the Jiuduansha Islands and Chongming Dongtan, respectively (Wang 2011). As a dominant species, *S. alterniflora* profoundly affects local biodiversity and economic development in the Yangtze River estuary (Li et al. 2009).

To support decision making, the Shanghai government has funded a series of scientific projects to study ecological effects of *S. alterniflora* invasion in the Yangtze River estuary and to develop strategies for controlling the invasion since 2004. Here, we briefly review how *S. alterniflora* invades, how the invasion affects native ecosystems, and the progress that has been made in controlling this invasive plant in the Yangtze River estuary. Most information described here can be found in our and other's published papers (e.g., Chen et al. 2007a, b; Gan et al. 2009; Jiang et al. 2009; Li et al. 2009, 2013, 2014; Liao et al. 2007; Ma et al. 2014; Nie et al. 2010; Wang et al. 2010; Xiao et al. 2011; Yan et al. 2008; Yuan et al. 2014; Zhang et al. 2006, 2010).

31.2 Mechanisms of *S. alterniflora* Invasion

S. alterniflora can employ several strategies for rapidly colonizing and expanding in new range. The plant can tolerate hypersaline environments in intertidal zones because its thick leaves contain salt glands to excrete excess salts (Wang et al. 2006a). The plant also has well-developed aerating tissues, which enable it to avoid waterlogging and provide sufficient oxygen for root growth (Mendelssohn and Postek 1982). As a C_4 plant, *S. alterniflora* has a higher net photosynthesis rate than C_3 native plants, such as *Phragmites australis* and *Scirpus mariqueter* (Jiang et al. 2009). Moreover, this invasive plant can quickly reproduce by both rhizomes and seeds. For example, an individual is able to horizontally extend to 226 cm and produces more than 600 mature seeds within one year (Zhang et al. 2006).

In addition, *S. alterniflora* has a greater competitive ability than native *S. mariqueter* and *P. australis* (Wang et al. 2006b; Zhao et al. 2008). In intertidal marshes, *S. alterniflora* promotes sedimentation rate and alter low-elevation habitats which are favored by *S. mariqueter* (Liao et al. 2007), leading to the low growth and reproduction of *S. mariqueter* (Chen et al. 2004a; Wang et al. 2010). Native *P. australis* in the *S. alterniflora*-invaded saltmarshes has lower survival rate, ramet density, plant height and aboveground biomass than in its own monoculture (Li et al. 2013). Moreover, as *S. alterniflora* has a high nitrogen fixation activity in both rhizosphere and litter (Liao et al. 2008), the plant N and soil inorganic N pools are higher in the invaded marshes (14.39 and 3.16 g N m^{-2}, respectively) than in native *P. australis* marshes (11.61 and 2.29 g N m^{-2}, respectively) (Peng et al. 2011).

Fig. 31.2 A seawall is being built in the Yangtze River estuary (From Ma et al. 2014)

In addition, human activities can speed up *S. alterniflora* invasion. For example, the disturbance due to massive seawall construction (Fig. 31.2) creates empty niches, which may facilitate colonization and expansion of *S. alterniflora* (Ma et al. 2014). Industrial and agricultural productions can also increase nutrient availability and thus enhance the invasion (Li et al. 2009).

31.3 Ecological Consequences of *S. alterniflora* Invasion

31.3.1 Effects on Native Plants

S. alterniflora rapidly outcompetes and even causes local extinction of native *S. mariqueter* (Fig. 31.3) (Chen et al. 2004b). *S. alterniflora* also threatens native *P. australis* in brackish ecosystems (Wang et al. 2006a). Recently, our results suggest that *S. alterniflora* can indirectly cause dieback of *P. australis* by transferring an endophytic, pathogenic fungus *Fusarium palustre* (Li et al. 2013, 2014). In North American salt marshes, this fungus leads to dieback of *S. alterniflora* (Elmer and Marra 2011). In the invaded Chongming Dongtan wetland, however, *S. alterniflora* grows healthily while its neighbor native plant *P. australis* shows obvious dieback syndrome due to *F. palustre* (Fig. 31.4) (Li et al. 2014). Further analyses indicate that *F. palustre* is likely transported by *S. alterniflora* from the native North American salt marshes into China. Thus, *S. alterniflora* reduces *P. australis*'s fitness in the invaded areas by acting as the vector and reservoir for pathogen shared with the native species (Li et al. 2013).

Fig. 31.3 *Spartina alterniflora* outcompetes *Scirpus mariqueter* in the Yangtze River estuary, China (From Weber and Li 2008)

Fig. 31.4 *Phragmites australis* dieback (in *grey* and *white*) caused by *Spartina alterniflora* (in *green*) invasion in the Yangtze River estuary, China

31.3.2 Effects on Microorganisms

S. alterniflora invasion aletrs bacterial community structure and diversity in the Yangtze River estuary (Wang et al. 2007; Nie et al. 2010). One of our studies has shown that the invasion of *S. alterniflora* affects some important functional microorganisms, such as the sulfate-reducing bacteria (SRB) which is responsible for sulfate reduction (Nie et al. 2009). *S. alterniflora* keeps higher richness and abundance of SRB in its rhizophere soil from the vegetative growth to senescent stage. However, the higher richness and abundance of SRB in the rhizospheres of native plants mainly occurr when the plants are in vegetative growth and reproductive stages. Such variation of SRB is closely associated with the changes of plant community composition which is caused by the invasion of *S. alterniflora*.

31.3.3 Effects on Soil Nematodes

Nematodes are the most abundant soil metazoans (Yeates 1999). In a study conducted in the Yangtze River estuary, a total of 48 genera of nematodes are found, belonging to six trophic functional groups (plant feeder, omnivore, fungal feeder, predator, algal feeder, and bacterivor) (Chen et al. 2007a, b). Because the invasion of *S. alterniflora* alters litter quality, relative abundance of bacterial-feeding nematodes (bacterivores) in soil is significantly increased by *S. alterniflora*, whereas that of plant- and algae-feeding nematodes is reduced by the invasion (Chen et al. 2007b).

31.3.4 Effects on Macrobenthos

Changes in ecosystem productivity and root system due to *S. alterniflora* invasion can alter soil physical and chemical properties in ways that influences benthonic invertebrate community (Table 31.1). For example, *S. alterniflora* changes abundance of some species such as *Assiminea violacea* and distribution of trophic functional groups, while *S. alterniflora* has no effect on overall density of macrobenthic

Table 31.1 Mean densities of five species of macrobenthonic invertebrates in native (*Scirpus mariqueter*) and invaded (*Spartina alterniflora*) communities

	Mean density (individuals m^{-2})	
Taxon	*Scirpus* community	*Spartina* community
Gastopodia		
Assiminea violacea	1351 ± 181	2086 ± 225
Assiminea lutea	249 ± 57	108 ± 36
Cerithidea sinensis	211 ± 41	81 ± 21
Stenothyra glabra	332 ± 83	54 ± 16
Lamellibranchia		
Glaucomya chinensis	163 ± 45	49 ± 12

All differences between the two plant communities are significant at $P < 0.05$ (Data extracted from Chen et al. 2005).

Fig. 31.5 The native tussock moth, *Laelia coenosa*, becomes a dominant insect species on *Spartina alterniflora* in the Yangtze River estuary, China

invertebrates (Chen et al. 2005). In addition, *S. alterniflora* provides more suitable food resources and habitats for crabs compared with native plants (Wang et al. 2008).

31.3.5 Effects on Above-Ground Arthropods

Because arthropods depend on foods and habitats provided by plants, any changes in plant composition may greatly affect arthropod community. For example, *S. alterniflora* significantly reduces the total abundance of insects because most native herbivory insects still prefer to native plants rather than *S. alterniflora* (Wu et al. 2009). The tussock moth *Laelia coenosa*, however, becomes increasingly prevalent on *S. alterniflora* over the past 5 years in the Chongming Dongtan wetland (Fig. 31.5) (Ma et al. 2015; Ju et al. 2016).

31.3.6 Effects on Birds

The Yangtze River estuary provides important stopover sites for migratory shorebirds on the East Asian-Australasian flyway. The Chongming Dongtan wetland has approximately 290 species of birds from 17 orders. Among them, 20 species are listed in the *China Red Data Book of Endangered Animals*, and 156 and 54 species are listed in the inter-governmental agreements between China and Japan, and between China and Australia for protecting migratory birds and their habitats, respectively. In each winter, more than one million individuals of the waders, geese, ducks, umbrette, gulls, and cranes overwinter in the Chongming Dongtan (data obtained from http://www.dongtan.cn). However, birds' habitats and shelters are being directly or indirectly damaged by *S. alterniflora* invasion because *S. alterniflora* forms dense stands that restrict bird movements and reduces food resources for many birds (e.g., geese, ducks and cranes) (Gan et al. 2009; Ma et al. 2011). However, *S. alterniflora* invasions may also favor certain birds such as Marsh Grassbirds (*Locustella pryeri*) (Ma et al. 2013).

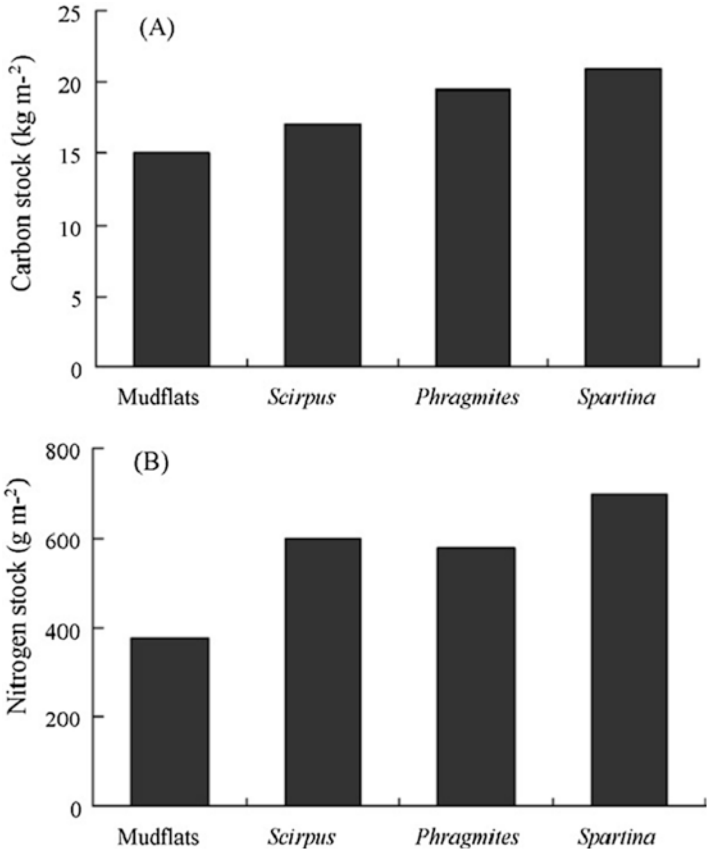

Fig. 31.6 Total carbon and nitrogen stocks in mudflats and in three plant communities (*Spartina alterniflora*, *Scirpus mariqueter* and *Phragmites australis*) in the Jiuduansha wetland of the Yangtze River estuary. The total stock is the sum of plant and soil stocks (From Liao et al. 2007; Li et al. 2009)

31.3.7 Effects on Ecosystem Processes

Changes in dominant primary producers can alter soil carbon and nitrogen cycling. Compared to native plants, *S. alterniflora* has greater standing biomass and net primary production resulting from higher leaf area index, net photosynthetic rate and longer growing season (Jiang et al. 2009). *S. alterniflora* also produces more litter that decays more slowly than native litter (Liao et al. 2007). In addition, *S. alterniflora* fixes substantial quantity of nitrogen even during litter decomposition (Liao et al. 2007). Moreover, *S. alterniflora* takes up more nitrogen from the tides than co-occurring native plants (Peng et al. 2011). Consequently, total carbon and nitrogen stocks (Cheng et al. 2006; Zhang et al. 2010) and carbon fluxes (Yan et al. 2008) in the Yangtze River estuary are higher in *S. alterniflora*-invaded area than in non-invaded area (Fig. 31.6) (Liao et al. 2007). In addition, methane emission is increased by *S. alterniflora* invasion (Yuan et al. 2014).

31.4 Management of *S. alterniflora*

S. alterniflora is being ecologically controlled in the eastern salt marshes of China, especially in the Yangtze River estuary (Li et al. 2009). Since 2006, Shanghai government has been attempting to control *S. alterniflora* invasions in the Chongming Dongtan wetland. Recently, Shanghai government is spending more than 1.3 billion RMB to control *S. alterniflora* invasion and restore habitats for migratory birds (Ju et al. 2012). This is perhaps the largest ecological restoration project for controlling a single invasive plant in the world. The integrated management includes cofferdam construction, clipping, flooding, sun-curing, planting native plants, and optimising water level (Tang et al. 2009; Tang et al. 2010; Xiao et al. 2011). By the end of 2012, more than 4000 m–length cofferdams have been constructed and the controlled area 133.3 ha. The project effectively inhibits *S. alterniflora* expansion and creates suitable habitats for migratory birds (Fig. 31.7). In addition, native *S. mariqueter* is being restored gradually through the project.

31.5 Concluding Remarks

The successful invasion of *S. alterniflora* in China is largely due to the combination of its intrinsic traits, properties of the invaded ecosystems and human disturbances. *S. alterniflora* can also indirectly cause dieback of *P. australis* by transferring a pathogenic fungus *F. palustre*. The invasion alters carbon and nitrogen cycling and seriously affect native organisms, including plants, microorganisms, soil nematodes, macrobenthos, arthropods, and birds in the Yangtze River estuary. Recent

Fig. 31.7 An part of the Chongming Dongtan wetland of the Yangtze River estuary, China, where an ecological engineering project is being implemented controlling *Spartina alterniflora* invasion and restoring the habitats of migratory birds

ecological restoration project for controlling *S. alterniflora* invasion is an effective way to restore native habitats. Further studies are needed to find an efficient way to control *S. alterniflora* invasion on a larger scale.

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Chapter 32
Phytophthora sojae

Xiaoren Chen and Yuanchao Wang

Abstract Oomycetes including *Phytophthora* species are eukaryotic organisms that morphologically resemble filamentous fungi, but are phylogenetically related to diatoms and brown algae in the Stramenopiles. Most of *Phytophthora* species are notorious plant pathogens that are responsible for the great damage on ornamentally and agriculturally important plants. One species, *P. sojae*, is the causal agent of root and stem rot in soybean and has been reported throughout most soybean-growing regions globally. In China, it has been classified as one of threatening quarantine pests due to the potential high risk to our soybean production, although some areas have already observed its presence. Here, we first provide an overview of the spread, distribution and impact of *P. sojae* in China. Then, the basis of invasiveness of the species into China is discussed, including its host range, life style, favorable environmental factors and molecular arms. We also summarize the control strategies of *P. sojae* in China, including quarantine and surveillance measures, pathogen detection methods, soybean resistance utilization, chemical application, biological and agricultural control methods. In the end, future work related to this species is suggested. We hope that the knowledge presented here can help us design better control strategies for this pathogen in China.

Keywords Oomycetes • *Phytophthora sojae* • Distribution • Impact • Risk analysis • Adaptation mechanism • Control strategy

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32.1 Introduction

Oomycetes form a unique lineage of eukaryotic organisms that morphologically resemble filamentous fungi but are phylogenetically related to diatoms and brown algae in the kingdom Straminipila (Thines 2014). Oomycetes include a large number of plant pathogens that cause extensive losses in agriculture and widespread degradation in natural plant communities. They include more than 100 species of the genus *Phytophthora* (Kroon et al. 2012). Two of the representative species in this genus are *Phytophthora infestans* and *Phytophthora sojae*. The best known potato late blight disease, which is caused by *P. infestans*, is responsible for the Irish potato famine in 1840s, and still results in considerable loss of potato crop yield worldwide each year (Erwin and Ribeiro 1996; Judelson et al. 2009; Kamoun et al. 2015). *P. sojae*, a hemibiotrophic and homothallic oomycete, has a narrow host range and its economic damage is limited to soybean (*Glycine max* (L.) Merr.) although it was also reported as a pathogen of other kinds of plants (Erwin and Ribeiro 1996; Tyler 2007; Kamoun et al. 2015). *P. sojae* was first reported as the cause of root rot disease of soybean in the state of Indiana of USA in the 1948 (Kaufmann and Gerdemann 1958). It has since then become an important soybean pathogen in soybean-producing areas in Asia, Africa, Australia, Europe, and North and South America (Erwin and Ribeiro 1996; Schmitthenner 1999). The pathogen causes damping off of seedlings and root and stem rot of older soybean plants (Fig. 32.1), and has been a limiting factor to successful soybean production with an estimated $1–2 billion loss per year worldwide (Tyler 2007).

Soybean, native to Northeast China, is an important source of protein for human and animal nutrition, as well as a major source of vegetable oil. China has a large number of soybean germplasm resources and the soybean industry has had a long history, with a solid reputation in the world. China is currently the fourth largest producer of soybean in the world, with planting area of about 8.8 million hectares and yield of 14.5 million tons in 2010, behind USA, Brazil and Argentina (Li and Bi 2012; Xue 2013). Due to the macroeconomic control and policy changes, in recent years the yield of soybean has decreased dramatically in China, leading to our critically increasing requirement for its importation from those three major soybean

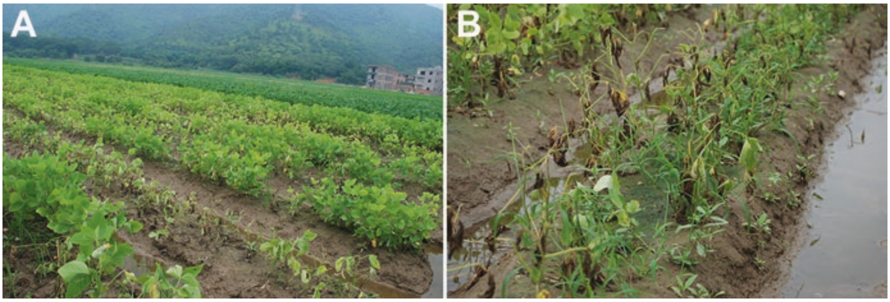

Fig. 32.1 Disease symptoms caused by *P. sojae* on soybean. (**a**) Damping off of seedlings in the field. (**b**) A closer look at the dying plants

production countries in which *P. sojae* has already widely populated (Li and Bi 2012; Xue 2013). In 2012, the soybean net imports from the above three countries hit 60 million tons (Li and Bi 2012). As a hazard organism to soybean, a strain or race of *P. sojae* with different genetic background present in other countries but not in China could establish in China from imported soybean and cause an unwanted impact.

In this chapter, we firstly review the spread, distribution and impact of *P. sojae* in China. Secondly, the basis of invasiveness of the species into China is discussed, including its host range, life style, molecular arsenal and favorable environmental factors. Thirdly, we summarize the control strategies of *P. sojae* in China, including quarantine and surveillance, pathogen detection, soybean resistance utilization, chemical application, biological and agricultural control methods. In the end, future work associated with this species is suggested.

32.2 Spread, Distribution and Impact of *P. sojae* in China

Root rot disease of soybean caused by *P. sojae* was first recognized in Northeast China in late 1980s (Su and Shen 1993). Since then, the incidence of this disease has been increasing in soybean-producing areas in China. So far, the pathogen has been sporadically found in the Inner Mongolia Autonomous Region, Fujian Province, Xinjiang Uygur Autonomous Region, Huanghe-Huaihe River Basin and Yangtse River Basin (Fig. 32.2) (Wen and Chen 2002; Zhu et al. 2003; Chen et al. 2004; Liu et al. 2006a; Wang et al. 2006a; Xiao et al. 2011). The genetic diversity studies indicated that the *P. sojae* populations found in Xinjiang and Inner Mongolia might be descendants of the Heilongjiang (Northeastern China) populations, while the populations in other areas (such as Fujian) might be immigrated from outside China (Wang et al. 2006b, 2007, 2009; Xiao et al. 2011). It has been suggested that *P. sojae* originated in North America as a pathogen of naturally occurring lupins before soybean was introduced into the land (Förster et al. 1994; Erwin and Ribeiro 1996; Kamoun et al. 2015). The incursion of *P. sojae* is a typical example of what can happen when an alien pest arrives into a new area. Quarantine and surveillance are therefore the best prevention methods. The Ministry of Agriculture of the People's Republic of China has evaluated the nature and possible effect on people, the environment, and the economy of the emerging *P. sojae* that is associated with soybean production, and officially identified it as one of the quarantine pest since 2007 (http://www.aqsiq.gov.cn/; June 22, 2016).

P. sojae presents a serious threat to Chinese soybean production, with potential yield losses from 10 to 100% (Xiao et al. 2011). Hence, importing soybean into China for consumption can cause critical biosecurity risks to our soybean cultivation. In addition, the increasing incidence of the disease has warned us about the high risks of the domestic spread of *P. sojae* found in several areas into pathogen-free soybean-growing areas in China. In soybean growing areas of the northern Midwest of the USA (which has a similar climate to that of China soybean growing areas), annual losses due to *P. sojae* can be as high as $200 million (Tyler 2007).

Fig. 32.2 The distribution of *P. sojae* in mainland China (so far in 2015)

Significant economic consequences could result in China if *P. sojae* was to spread from infested areas to pathogen-free production areas in either of cases.

32.3 The Basis of Invasiveness of the Species into China

Under risky circumstances, it is essential for us to fully understand the likelihood of the pathogen establishing in different areas of China and undertake the risk analysis. The knowledge learned from the studies on the mechanisms of establishing of alien strains or races of the pathogen in ecological environment conditions in China will help dissect the prevalence mechanism of the disease and therefore develop effective control strategies for securing our soybean industry.

32.3.1 Host Ranges

P. sojae has a narrow host range. Soybean is the only economically important host. About 26 species of the genus *Lupinus* (lupins) have also been reported as hosts (Erwin and Ribeiro 1996; Tyler 2007; Kamoun et al. 2015). Laboratory studies confirm that lima bean (*Phaseolus lunatus*), string bean (*Phaseolus vulgaris*) and cranesbill (*Geranium carolinianum*) are susceptible to *P. sojae* (Erwin and Ribeiro 1996; Kamoun et al. 2015). Among the alternative hosts, around 12 species of the genus *Lupinus* (*L. albus*, *L. angustifolius*, *L. digitatus*, *L. hartwegii*, *L. incanus*, *L. luteus*, *L. micranthus*, *L. mutabilis*, *L. nanus*, *L. perenius*, *L. polyphyllus* and *L. pubescens*) have been already introduced from abroad into China and cultivated across the country. The other potential host plants are widely distributed in China (Flora Reipublicae Popularis Sinicae; http://frps.eflora.cn/; June 22, 2016).

P. sojae has been continuously observed entering China on shipments of soybean from major soybean-growing countries (the reports from General Administration of Quality Supervision, Inspection and Quarantine of China; http://www.aqsiq.gov.cn/; June 22, 2016). Should soybean infected with the species be imported into China or transferred within domestic planting areas, infested soybean could act as a vehicle for exposure of the organism in the environment, as the growing season for soybean and other host plants in China (spring and summer) would be expected to coincide with the most likely period of importing or dispatching soybean. The risk of infection of soybean and alternative host plants is likely to be quite high if contaminated soybean is spread into the pathogen-free areas. The likelihood estimate is high that spread to commercial production areas would result in unwanted economic and environmental consequences to China.

32.3.2 Life Style

Like other *Phytophthora* species, *P. sojae* grows primarily as aseptate hyphae. *P. sojae* is heterotrophic and saprophytic and grows readily in the presence of an organic substrate. The pathogen thrives in low-lying or wet field conditions, and in compacted or heavy clay soils. *P. sojae* is capable of growing in the temperature range of 5–35 °C, the optimum being in the range of 25–30 °C (Erwin and Ribeiro 1996; Tyler 2007).

Two kinds of asexual cell types are commonly produced in water, zoosporangia (Fig. 32.3a–b) and zoospores. Zoosporangia can germinate and infect host tissues directly, or they can release 10–30 motile, flagellate and wall-less zoospores. Zoospores are able to swim in water for several hours and can swim chemotactically toward soybean root exudates (Morris and Ward 1992). Physical infection occurs when the zoospores encounter the plant surface and transform into an adhesive cyst that germinates within 20–30 min to produce a germ tube that penetrates the plant. Using a *GFP*-transgenic isolate, Dr. Chen and colleagues in Dr. Yuanchao Wang's lab discovered that the germ tube length of cysts germinating on the roots of resistant soybean cultivar was longer than that on the roots of susceptible cultivar (Chen et al. 2009). Penetration occurs within 1 h of germination (Tyler 2007). All parts of the soybean plant can be infected by *P. sojae*, from germinating seedlings to mature plants. In the field, soybean infection typically initiates below ground and subsequently produces spreading cankers that destroy root tissues or travel up the stem (Kamoun et al. 2015). *P. sojae* can

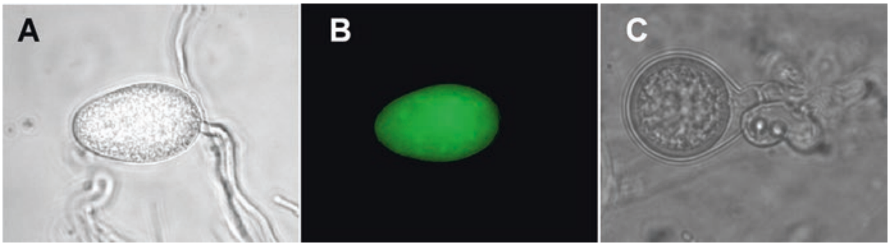

Fig. 32.3 Reproduction organs of asexual zoosporangium and sexual oospore of *P. sojae*. A sporangium of a *GFP*-transgenic isolate of *P. sojae* (Chen et al. 2009) was observed under bright field (**a**) and ultraviolet excitation (**b**). (**c**) An oospore forming in an oogonium laterally mated with an antheridium

cause damping off of soybean seedlings, and root and stem rot of established plants (Fig. 32.1). Leaves can be infected in the field as a result of rain splash of propagules. Damping off affects germinating seeds or emerged seedlings. Established plants can become infected when the soil is saturated for prolonged periods, especially if the soil is poorly drained. Disease is favored by warm (25–30 °C) and wet conditions, and the asexual epidemiology is often explosive during the crop growing season.

Under unfavorable conditions, homothallic *P. sojae* produces abundant, thick-walled sexual oospores (Fig. 32.3c). Because oospores are thick-walled, they are resistant to desiccation, cold temperatures, and other extreme environmental conditions, and can survive in the soil, in the absence of a host plant, for a long period of time, which therefore makes it more difficult to control the disease (Erwin and Ribeiro 1996; Tyler 2007). In the following spring when soil moisture is at field capacity, the oospores germinate and produce abundant zoosporangia which can germinate directly to produce hyphae or else produce zoospores.

32.3.3 Environmental Factors

Most of the territory of China lies in the temperate belt. China's climate is characterized by two distinct types, the continental monsoon climate and the complex climate. The precipitation in China varies markedly between the four seasons, with rain falling mostly in summer, and is distributed very unevenly from region to region. Topographically, China slopes from the west to the east, forming three distinct terraces. Mountainous regions, hilly areas and plateaus comprise 66% of the total territory. In terms of climate, China may be divided between the humid eastern region and the dry west. The humid east may be further subdivided between the warm and humid south and southeast and the temperate-to-cool, moderately humid north and northeast. Much of the humid eastern region of China exhibits a monsoonal pattern of temperature and precipitation. In a monsoon climate, the warm summer months are typically the months of maximum precipitation (National

Coordination Committee on Climate Change 2004, http://www.ccchina.gov.cn/file/en_source/da/da2004110901.pdf; June 22, 2016).

Soybean, native to China, has been grown in almost all of provinces of China (Pu and Pan 1982; Wang and Gai 2000). In 2010, the soybean planting area in China is about 8.8 million hectares, producing about 14.5 million tons of soybean (Li and Bi 2012; Xue 2013). The provinces with large planting area include Northeast provinces (Heilongjiang, Jilin and Liaoning), Shandong, Henan, Hebei, Tianjin, Jiangsu, Anhui, Zhejiang, Fujian, Guangdong, Guangxi, Sichuan, Hunan and so on. Heilongjiang, Jilin and Liaoning are the leading soybean growing provinces of China (Li and Bi 2012; Xue 2013). In these areas, environmental conditions in soybean-growing season are favorable for infection by *P. sojae* zoospores ranged between 27 and 32 °C and damping off ranged between 20 and 30 °C (Erwin and Ribeiro 1996).

Based on the above information, it can be predicted that the likelihood is high that *P. sojae* would establish in almost every soybean-growing place in China. Several factors would influence the prevalence of the species in soybean in the field and thus impact on the likelihood of the disease outbreak. Firstly, there is opportunity for infection because *Phytophthora* species are able to survive between crops as oospores in and on host plant debris in the soil. In the soybean planting areas in China, environmental conditions are favorable for survival of *P. sojae* oospores. Secondly, *Phytophthora* species are able to germinate and infect host tissues directly, or it can release zoospores, which can then infect the plant. In soybean planting areas, rain falls mostly in summer, which is very favorable for the sporangium formation and dispersing of swimming zoospores. Thirdly, environmental conditions conducive for soybean production in China are also conducive for infection of soybean by the species. For example, optimum temperatures for soybean growth range between 18 and 26 °C. Average climatic data for the soybean producing areas indicates that prior to (May to August) and during harvest (September to October), average temperature ranges and precipitation levels are conducive for infection by the species. Annual rainfall is most abundant during April to September (China Meteorological Administration; http://http://www.cma.gov.cn/; June 22, 2016), which favors the spread of the pathogen in the form of swimming zoospores during the crop growing season.

In view of all the points, the likelihood of *P. sojae* establishing and spreading in soybean-growing areas in China is considered high and is therefore non-negligible.

32.3.4 Breaking-in Arms

In 1997, Enkerli and colleagues characterized the ultrastructure of *P. sojae* infection of soybean roots, and revealed that the penetration by the pathogen can be accomplished within hours (Enkerli et al. 1997). Within days, infected plants would start to collapse and die if no effective measures were taken, leading to essential economic losses (Erwin and Ribeiro 1996). Increased awareness of the impact on soybean production has driven researchers around the world to focus on the studies of mechanisms of *P. sojae* virulence, host resistance, and the molecular basis of recognition between *P. sojae* and soybean (Tyler 2007; Dorrance and Grünwald 2009; Gijzen and Qutob 2009; Kamoun et al. 2015).

32.3.4.1 Effector Proteins

Genomic information and gene expression data have been obtained to understand the pathogen and/or the interaction with the hosts (Qutob et al. 2000; Tyler et al. 2006; Torto-Alalibo et al. 2007; Wang et al. 2011; Ye et al. 2011). Evidence has been increasing that oomycetes including *P. sojae* probably yield a large number of effector proteins that collectively interfere with plant life in order to create a favorable environment for pathogen infection (Stassen and Van den Ackerveken 2011). Oomycetes secrete effectors that can be active outside of host cells (apoplastic space), for example inhibiting host defense enzymes, or inside host cells (cytoplasmic space) where they can interfere with plant physiological functions, in particular suppression of defense (Kamoun 2006; Wang et al. 2011; Stassen and Van den Ackerveken 2011).

Various types of protein families with functions in *P. sojae* virulence were also discovered by Chinese researchers, including transcription factor (Ye et al. 2013; Sheng et al. 2015), mitogen-activated protein kinases (Li et al. 2014; Gao et al. 2015), TatDnuclease (Chen et al. 2014), toxin-like proteins such as the NLP family (Qutob et al. 2006; Dong et al. 2012), and two large, rapidly diversifying classes of effector proteins (RXLR and CRN effectors) (Tyler et al. 2006; Jiang et al. 2008; Haas et al. 2009) that can translocate inside host plant cells (Dou et al. 2008; Kale et al. 2010). To understand the roles of RXLR effectors, Dr. Wang's lab performed a systematic functional characterization of a large sample of these Avh proteins encoded in the *P. sojae* genome. It was revealed that most of the proteins have the potential to suppress plant cell death triggered by effectors and a PAMP, and the expression of these effectors during *P. sojae* infection is dominated by a small number of proteins whose program of expression appears coordinated to maximize suppression of host defenses (Wang et al. 2011). The majority of oomycete RXLR effectors have little similarity, at the primary amino acid sequence level, to known proteins and many are thought to be too small to encode enzymatic activities. However, Dong *et al.* in Dr. Wang's lab for the first time discovered that a *P. sojae* a virulence RXLR effector Avr3b is a secreted NADH and ADP-ribose pyrophosphorylase that modulates plant immunity (Dong et al. 2011). Avr3b is so far the only one oomycete RXLR effector encoding a protein with an enzymatic activity. Recently, Dr. Daolong Dou's group found that a *P. sojae* isochorismatase effector, along with its *Verticillium dahlia* homologue, disrupts the plant salicylate metabolism pathway to suppress host immunity (Liu et al. 2014a, b). The discovery of these unconventionally secreted enzymes in both fungi and *Phytophthora* suggests that this may be a central mechanism of virulence in filamentous plant pathogens. Such remarkable progress on oomycete effector biology will lead to novel and effective strategies for plant disease management.

32.3.4.2 Racial Variation

Genetic inheritance studies using molecular markers led to discoveries of high-frequency gene conversion (also known as loss of heterozygosity) (Chamnanpunt et al. 2001) and epigenetic silencing (Qutob et al. 2013) that are probably important mechanisms underlying the variation in *P. sojae* populations. To develop the effective control strategies for the disease, it is vital to understand the racial diversity of *P. sojae* in soybean-producing regions.

The virulence pathotypes of North American *P. sojae* races on a set of eight differentials with *Rps* ("resistance to *P. sojae*") genes (*Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps3a*, *Rps6*, or *Rps7*) were summarized in 2004 (Grau et al. 2004). At least 55 physiologic races of *P. sojae* have been identified in the USA on the basis of the unique reaction patterns produced on the differential soybeans genotypes. The dominant races and racial diversity are found to differ in each area in the USA (Grau et al. 2004). In Japan, six Japanese differential cultivars were selected to characterize the Japanese isolates (Tsuchiya et al. 1990), and so far 15 virulence pathotypes (races A, B, C, D, E, F, G, H, I, J, K, L, M, N and O), different from physiologic races of North America, have been identified (Tsuchiya et al. 1990; Sugimoto et al. 2010b).

Near-isogenic lines (NILs), each carrying one of 14 *Rps* genes in the background of "Williams" (*rps*; "susceptible to *P. sojae*") were developed by Dr. Richard Bernard at the USDA-ARS (Urbana, IL, USA) (Table 32.1), and have been used for the identification of resistance soybean germplasm and of virulence formula of *P. sojae* isolates in China. Abundant virulence diversity has been detected in *P. sojae* populations in China, especially in the ones from Yangtse River Valley (Zhu et al. 2003). In 2011, it was reported that virulent frequency of 115 Chinese isolates from

Table 32.1 *Phytophthora* resistance genes in soybean

Differential host	*Rps* gene	Molecular linkage group
Harlon	*Rps1a*	N
Harosoy13XX	*Rps1b*	N
Williams79	*Rps1c*	N
PI103091	*Rps1d*	N
Williams82	*Rps1k*	N
L76-1988	*Rps2*	J
Chapman	*Rps3a*	F
PRX146-36	*Rps3b*	F
PRX145-48	*Rps3c*	F
L85-2352	*Rps4*	G
L85-3059	*Rps5*	G
Harosoy 62XX	*Rps6*	G
Harosoy	*Rps7*	N
Williams[a]	–	F

[a] Williams does not contain any resistance gene to *P. sojae*

Huanghuai Region was not more than 10% on differential hosts which carry *Rps1a*, *Rps1c*, and *Rps1k* genes (Tang et al. 2011). In contrast, the composition of *P. sojae* virulence genes in the isolates from the major soybean-growing area, Heilongjiang Province, was recently found very complex and more than 10% of the isolates had overcome the resistance conferred by the strong resistance gene *Rps1k* (Ma and Han 2013). However, several studies focusing on the race distribution in Heilongjiang Province found that race 1 has been the most predominant race for years since 1999 (Zhang et al. 2008; Shen et al. 2010; Xu et al. 2011; Ma and Han 2013).

Although much effort has been taken, the race distribution of *P. sojae* in infested areas in China is still unclear. The reaction patterns that most of Chinese isolates produced on the differentials did not correspond to those of most of the 55 races previously identified in the USA. Chinese differential cultivars should be selected to characterize the Chinese isolates (Xu et al. 2002). One area of future research should define in detail the virulence pathotypes and dominant races of *P. sojae* in China.

32.4 Control Strategies of *P. sojae* in China

32.4.1 Quarantine and Surveillance

In China, soybean import is overseen by the Entry-Exit Inspection and Quarantine Bureau of the People’s Republic of China (CIQ). CIQs are the government compliance organization working to regulations enacted by the General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China (AQSIQ). There are two important regulations governing the soybean import, “Law of the People's Republic of China on the Entry and Exit Animal and Plant Quarantine” and “Regulations for the Implementation of the Law of the People's Republic of China on the Entry and Exit Animal and Plant Quarantine”. The production and processing companies overseas are responsible for developing their own control system that regulates chemical and pest control activities and ensures compliance with Chinese phytosanitary requirements and the regulations. CIQ import permit registration must be completed for the foreign companies before soybean is transported. The consignment is in compliance with phytosanitary requirements described in the protocols of phytosanitary and inspection requirements for the export of soybean into China between China and the exporting country, and does not contain the quarantine pests of concern to China.

Although the pathogen is classified as a hazard in China, specific phytosanitary measures are few. Once the pathogen is detected in an alien consignment, the infested goods would be destroyed immediately (Li et al. 2013). Other methods are not available at the dock of the customs. Tests have been made by Chinese researchers to the commonly used fumigants including methyl bromide, propylene oxide, methyl iodide and carbonyl sulfide to determine the actual level of efficacy of the treatments (Li et al. 2013). However, these fumigants have not been permitted to be used in practice for the control of the species.

Once the pathogen is detected inside China, the cases should be reported to the plant quarantine departments immediately, and timely measures must be taken to eradicate the pathogen. All the infected or potentially infected plants in the field must be destroyed. Potentially contaminated plants and soil, tools are prohibited to be transferred outside to prevent spreading of the pathogen. An isolation belt between the infested and non-infested areas must be established to prevent the extending of the plants. Due to the fact that the thick-walled sexual, resting oospores can survive in the soil in the absence of a host plant for many years, paddy-upland rotation without involvement of soybean is recommended.

32.4.2 Pathogen Detection

With the increasing amount of soybean traded with different countries, rapid detection of *P. sojae* in the consignment and soil carried with the transported soybean is important not only for soybean trade between China and other countries, but also for controlling the spread of *P. sojae* within China. Therefore, rapid, cost-effective and efficient detection measures are required for the plant quarantine departments. In addition, it would reduce the risk of spread of this pathogen if rapid methods could be performed in the field to minimize the delay between sampling and diagnosis. Some methods available for *P. sojae* detection in China are listed in Table 32.2.

Initially, the pathogen detection was a time-consuming process including the pathogen baiting, isolation and morphological characterization (Klein 1959; Canaday and Schmitthenner 1982; Peng and Anderson 1998; Chen et al. 2004; Xiao et al. 2006; Liu et al. 2008). Because *P. sojae* is a soil borne pathogen, it is very important to monitor the presence of it in the soil residues carried by the transported soybean. Soybean leaf-disc baiting method, developed by Canaday and Schmitthenner (Canaday and Schmitthenner 1982), has been improved for better efficiency, especially by the addition of chemicals to inhibit the growth of other fungi (Wang et al. 2005), and applied to the detection of *P. sojae* in soil (Peng and Anderson 1998; Wang et al. 2005; Liu et al. 2008). Soils can be submerged into water and leaf discs floated on the surface to capture zoospores, then infested leaf discs can be plated on selective medium (containing pentachloronitrobenzene at 25 $\mu g \bullet ml^{-1}$, carbendazim at 25 $\mu g \bullet ml^{-1}$, rifampicin at 20$\bullet \mu g ml^{-1}$and ampicillin at 50 $\mu g \bullet ml^{-1}$) to let the pathogen grow onto the medium. However, soils which are dry or have been frozen require saturation followed by an incubation period of 1 to 2 weeks of continuous moist conditions and temperatures of 15–18 °C. This incubation period helps “break” dormancy of the oospores. Mesh screening method was also developed to collect and concentrate oospores from soil samples (Wang et al. 2006c). The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) staining can be utilized to determine whether the oospores recovered from the

Table 32.2 Commonly used methods for detection of *P. sojae*

Name	Brief description	Purpose	Comment
Leaf-disc baiting	Pathogen baiting and isolation from samples	Morphological characterization	Time-consuming, but most reliable method
Mesh screening	Samples were screened by a series of different sized meshes to collect and concentrate oospores from soil samples	Morphological characterization	Only for oospore concentration. time-consuming, but most reliable method
Serology assay	Samples were detected by ELISA using antiserum that was obtained with mycelial protein extract of the pathogen.	Detection of the pathogen presence	Costly and tech required
Isoenzyme assay	Electrophoresis and Isoenzyme pattern comparison with that of standard isolate	Detection of the pathogen presence	Costly and tech required
(q)PCR	PCR amplification using specific primers (Ypt, CoxII, ITS, transposon, elicitin, Avr, etc.)	Detection of the pathogen presence	High specificity and efficiency, but tech and equipment required and with the frequency of false-positives
Loop-mediated isothermal amplification (LAMP)	LAMP uses a set of four or six primers and the strand displacement activity of Bst DNA polymerase to amplify DNA under isothermal conditions, and products can be monitored visually.	Detection of the pathogen presence	High specificity and efficiency with lower equipment dependency, but tech required

soil are alive or dead (Sutherland and Cohen 1983; Wang et al. 2006c), which is helpful to the risk analysis.

With the development of biology technology, serology (Dou et al. 1997; Wen et al. 2002) and isoenzymes (Zhou et al. 1995; Wen 2003) have been applied to detection of *P. sojae* in China in recent years. Due to their inherited defects, such as high-tech equipment required, elaborate and complicated assay procedures, expensive reagents and time requirements, more rapid, sensitive and cost-effective methods for the specific detection of *P. sojae* are required. A rapid method was then developed on the basis of conventional polymerase chain reaction (PCR) using the species-specific primers (PS1/PS2) and becomes the most common method for detection of *P. sojae* in China (Wang et al. 2004, 2006c). The primers specific to *P. sojae* are based on the ribosomal internal transcribed spacer (ITS) regions, producing a 330-bp fragment (Fig. 32.4) (Wang et al. 2004, 2006c; Chen et al. 2005). Tests with *P. sojae* genomic DNA determined that the sensitivity of the primer set is

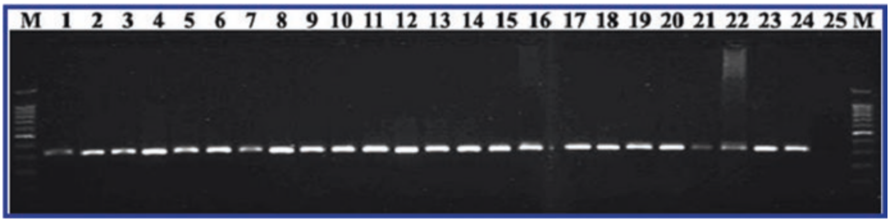

Fig. 32.4 Agarose gel electrophoresis of PCR products using the *P. sojae*-specific primers PS1 and PS2 (Wang et al. 2006c). PCR amplification products of 24 isolates of P. sojae (lanes 1 to 24) were collected from different sources as follows: Heilongjiang Province (lanes 1 to 12), United States (lanes 13 to 19), Brazil (lanes 20 to 22), Canada (lane 23), and Argentina (lane 24). Lane 25, no template DNA; M, 100-bp DNA ladder

approximately 1 fg (Wang et al. 2006c). The PCR assay, combined with the soil mesh-screening method, allowed the detection of *P. sojae* from soil within 6 h, with a detection sensitivity of two oospores in 20 g of soil (Wang et al. 2006c). Real-time fluorescent quantitative PCR assays were also developed to detect the pathogen directly in soil samples carried with soybean (Wang et al. 2006c). Other specific primers targeting different genes such as *Ypt1, coxII*, transposon and elicitin were also designed in Dr. Wang's lab.

In 2012, an alternative amplification method that can be used in the field to detect *P. sojae* without a thermal cycler was for the first time developed based on loop-mediated isothermal amplification (LAMP) of DNA in Dr. Wang's lab (Dai et al. 2012). The reported LAMP technique has the advantages of simple detection and rapid assay time (< 80 min) over other methods (Dai et al. 2012). The detection limit of the LAMP assay for *P. sojae* was 10 pg/uL of genomic DNA per reaction (Dai et al. 2012). However, an identifiable target specific to *P. sojae* (such as A3aPro, a 300-bp deletion element in the upstream (1.5 kb in the promoter region) of the virulence gene *Avr3a* in *P. sojae* Race 7) need to be determined prior to assays. Nevertheless, this novel method can be used for the rapid diagnosis of *P. sojae* in plants and in production fields, which in turn could make it possible to control the dispersion of the pathogen.

32.4.3 Resistance Utilization

Soybean is the only economically important host although several species of lupins and other plants have also been reported as hosts of the pathogen (Kamoun et al. 2015). Utilization of resistance varieties is the most economical and environmentally safe mean to manage plant pathogens (Agrios 2005). The effective strategy available to management *P. sojae* in soybean has primarily relied on breeding for resistance (Kamoun et al. 2015). Two distinct types of host resistance have been described: (1) race-specific resistance conditioned by a single dominant *Rps* gene and (2) partial resistance conferred by multiple genes acting together (Sugimoto et al. 2012).

Monogenic resistance conditioned by certain *Rps* genes has been providing reasonable protection against the majority of *P. sojae* populations in the USA for the last four decades (Sugimoto et al. 2012). To date, about 20 *Rps* genes/alleles on four chromosomes have been identified (Sun et al. 2014b), and *Rps1k* confers strong resistance against a large number of North American *P. sojae* races, and has been the most stable and widely used *Rps* gene in the last two decades (Sugimoto et al. 2012). More than 3500 USA commercial cultivars for their resistance to *P. sojae* were recently tested, and 51% were found to carry at least one *Rps* gene (Slaminko et al. 2010). Half of them have *Rps1c*, while another 40% of them have *Rps1k*-mediated resistance to *P. sojae* (Slaminko et al. 2010). Meanwhile, the effectiveness of 14 *Rps* genes against 109 *P. sojae* isolates from 14 regions in Japan was examined and *Rps1d, Rps1k, Rps8, Rps1a, Rps1c, Rps7, Rps3b* and *Rps1b* were found to provide resistance to 47–81% of the isolates (Moriwaki 2010). *Rps1d* and *Rps1k* were the most effective resistance genes (Moriwaki 2010). These results indicated that *Rps1d* and *Rps1k* are important sources of resistance that can be used to breed new resistant cultivars in the USA as well as in Japan (Sugimoto et al. 2012).

There are a large number of soybean germplasm resources in China, and a lot of germplasm resistant to *P. sojae* have been identified since 1990s (e.g. Wang et al. 2001a, b; Zhu et al. 2006; Sun et al. 2008; Ma et al. 2010; Shen et al. 2010; Wu et al. 2010; Xu et al. 2011). In 2003, it was found that *Rps1k* was the most effective resistance gene in Heilongjiang Province, followed by *Rps1c* (Zhu et al. 2003). In 2006, 120 soybean cultivars or lines from 11 provinces in China were evaluated for their responses to 10 strains of *P. sojae* and 110 cultivars or lines were identified conferring resistance to 1–10 strains (Zhu et al. 2006). One hundred and twenty cultivars or lines displayed 57 different reaction types when tested on those 10 strains (Zhu et al. 2006). In 2013, resistance of 261 soybean cultivars or lines growing in Heilongjiang Province to *Phytophthora* root rot (PRR) was evaluated (Ma and Han 2013). It was predicted that 30 soybean cultivars or lines contained *Rps* genes and 3 major genes *Rps1k*, *Rps3a* and*Rps1c* were probably distributed in ten cultivars (Ma et al. 2013). Due to the complex virulence formula of Chinese isolates and genetic background of soybean germplasm, it is yet unclear about the distribution of *Rps* genes in the soybean landraces in China.

Previous studies have shown that the germplasm growing in Huanghuai Region and Yangtse Valley contain higher resistance to the soil borne disease than the ones from other areas (Wang et al. 2001b; Zhu et al. 2006). Furthermore, there are features of a rich diversity of resources and resistance in soybean germplasm in Huanghuai Region (Wang et al. 2001a, b; Tang et al. 2011). Therefore, screening of resistant cultivars or lines for China soybean industry should be based on the germplasm from those areas. Recently, the resistance of 55 soybean cultivars or lines from 11 provinces to *P. sojae* was evaluated and it was postulated that the most effective *Rps* genes *Rps1a*, *Rps1c* and *Rps1k* are not widely distributed in soybean germplasm from Huanghuaihai Region (Tang et al. 2011). It was also found that some cultivars or lines and wild soybeans could possibly carry novel *Rps* genes that are effective to control the disease in China (Huo et al. 2005; Zhu et al. 2006; Xu et al. 2011). The novel *Rps* genes, such as *RpsYD25* from Yudou 25 (Fan et al. 2009) and *RpsJS* from Nannong 10-1 (Sun et al. 2014b), should be further fine mapped and isolated for characterization in the future work. These *Rps* genes together are potential sources of resistance that can be used to breed new resistant cultivars in China.

While single gene-mediated resistance has been an effective means for managing this soil borne disease, the value of partial resistance conferred by multiple genes should be recognized. Continuous utilization of race-specific *Rps* genes in soybean cultivars grown in North America has resulted in selection pressures that promote the evolution of more pathogenic races of *P. sojae* (Grau et al. 2004). Physiological races of *P. sojae* that can overcome the resistance conferred by single dominant *Rps* genes have been detected (Dorrance et al. 2003). Partial resistance, sometimes referred to as tolerance or field resistance, is characterized by disease progression at a much slower rate than what occurs in susceptible cultivars. Generally, partial resistance has been described as the relative ability of susceptible plants to survive infection without showing severe symptoms like death, stunting, or yield loss (Agrios 2005). It was reported that cultivars containing partial resistance are not severely damaged or even effective against all races of *P. sojae* in the field (Schmitthenner 1985; Dorrance et al. 2003). It was found that yields of soybean cultivars with partial resistance were not significantly different from those of cultivars with single *Rps* genes or *Rps* gene combinations (Dorrance et al. 2003). All the results demonstrated that partial resistance can provide protection when soybean plants are subjected to the infection by *P. sojae*. About half of the soybean cultivars growing in USA and Japan had high levels of partial resistance or tolerance to *P. sojae* (Sugimoto et al. 2012). These cultivars may also be useful as sources of resistance for breeding new cultivars adapted to other countries where *P. sojae* is a problem.

It has been found that the heritability of quantitatively inherited partial resistance is relatively high (Weng et al. 2007; Han et al. 2008). In China, molecular markers linked to *Rps* genes or quantitative trait loci (QTLs) underlying partial resistance, such as microsatellite (simple sequence repeat, SSR), randomly amplified polymorphic DNA (RAPD) and single nucleotide polymorphism (SNP), have been identified, and could be used for marker-assisted selection (MAS) to efficiently identify seedlings carrying the resistance genes (Han et al. 2006, 2008, 2013; Weng et al. 2007; Li et al. 2008; Fan et al. 2009; Zhao et al. 2014; Sun et al. 2014a, b). In 2006, a first effort was reported, aiming at identifying QTLs on the linkage groups which were associated with soybean PRR in a population from across between “Conrad”, a North American cultivar with high levels of partial resistance in the field, and “OX760-6-1”, a Canadian breeding line susceptible to PRR (Han et al. 2006). A few QTLs were found stable across different ecological conditions and would be useful for marker assisted-breeding (Han et al. 2006). Four QTLs underlying tolerance to PSR in F_6 and F_7 populations (“Conrad” ×“OX760-6-1”) consisting of recombinant inbred lines (RILs) using laboratory or field tests with Chinese isolates were identified (Weng et al. 2007; Han et al. 2008). Later, a total of 8 QTLs (QPRR-1 to QPRR-

8) for partial resistance to Chinese and Canadian isolates were identified on soybean molecular linkage groups (MLG) F, D1b+w, A2, B1 and C2 using a RIL population consisting of 140 $F_{2:5}$ and $F_{2:6}$ individuals derived from a cross between 'Conrad' and "Hefeng 25" (a Northeastern China cultivar tolerant to *P. sojae* infection) (Li et al. 2008, 2010). Two markers, Satt325 and Satt343 on MLG F, were located near QPRR-1. Markers Satt005 and Satt600 on MLG D1b+w were located near QPRR-2 while another two markers Satt579 and Sat_089 on MLG D1b+w were located near QPRR-3. These three QTLs derived their beneficial alleles from 'Canrad' and were located at chromosomal locations known to underlie PRR tolerance in diverse germplasm. Five QTLs derived beneficial alleles from "Hefeng 25" (Li et al. 2010). More QTLs for partial resistance to *P. sojae* are being identified (Sun et al. 2014a). The accumulation of tolerance loci is positively correlated with decreases in disease loss percentage (Li et al. 2010). The pyramid of loci underlying tolerance to PRR provides germplasm useful for crop improvement by marker-assisted selection and will provide durable cultivar tolerance against the soil borne disease.

32.4.4 Chemical Control

Oomycete pathogens, including *P. sojae*, are very difficult to control because they are unaffected by the majority of fungicides (Agrios 2005). The difficulty of control is worsened for most *Phytophthora* diseases because the host plants are often attacked underground where they cannot be economically treated (Erwin and Ribeiro 1996).

Early chemicals to control oomycete diseases such as late blight included "Bordeaux mixture", a broad-spectrum antimicrobial mixture containing copper salts (Agrios 2005). In the past 37 years or so, chemicals that exhibit specificity to oomycetes have been developed. An example of these chemicals is the phenylamide Metalaxyl, which displays excellent performance as systemic fungicides with protective, curative, and long-lasting activity against many important plant pathogenic oomycetes (Schwinn and Staub 1995). Since the introduction of Metalaxyl into the market in 1977, this versatile fungicide has been used extensively to control oomycete diseases including PRR (Schwinn and Staub 1995). Subsequently, the (*R*)-enantiomer of Metalaxyl (Mefenoxam, a Syngenta Trade Mark) was found to provide the majority of the biological activity against oomycetes (MacBean 2012). Soon after their widespread use, however, pathogen genotypes that were fully insensitive to the chemicals were identified for some oomycete species, but not *P. sojae* (Gisi et al. 2000; Lamour and Hausbeck 2000; Randall et al. 2014; Childers et al. 2015). Metalaxyl, along with Mefenoxam, is one of the widely used fungicide to control PRR in China now and so far no insensitive isolates of *P. sojae* to the chemicals have been identified (Jin et al. 2010; Cui et al. 2013). Since Metalaxyl acts highly selectively at a single target site, a mutation might easily lead to resistance by changing the target site affinity (Randall et al. 2014). Recent studies indicated that there is a high potential risk of Metalaxyl resistance of *P. sojae* after a long term application in China (Zuo et al. 2005; Yang et al. 2012; Cui et al. 2013; Guo et al. 2014).

More recently developed oomycete-specific chemicals include the family of carboxylic acid amide (CAA) fungicides (Lamberth et al. 2008). It is composed of three different groups of chemicals, namely cinnamic acid amides, valine amide carbamates and mandelic acid amides, and has been applied to control oomycete diseases worldwide (Zhu et al. 2010). Members of the group of cinnamic acid amides include Pyrimorph (Mu et al. 2007) and Flumorph (Liu and Liu 2002) that are synthesized and registered in China. Pyrimorph is currently registered for the control of *Phytophthora* blight of pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) late blight, while Flumorph is registered for controlling *P. infestans* on potato and tomato, *P. capsici* on pepper, *Pseudoperonospora cubensis* on cucumber, and *Plasmopara viticola* on grapevine in China (China Pesticide Information Network; http://www.chinapesticide.gov.cn; June 22, 2016). Tests showed that Flumorph can significantly inhibit the growth of *P. sojae in vitro*, and effect is better than that of Metalaxy (Liu et al. 1999; Kong et al. 2007). Both novel chemicals have the potential ability to control PRR in China. The phenylamide and CAA chemicals can be applied in the field in the form of plant spraying, seed coating agent or flowable concentrates for seed coating (Liu et al. 2006b; Chou et al. 2014).

Due to the complication that *P. sojae* appears to have a genetic flexibility which enables it rapidly to adapt to and overcome chemical control measures, fungicide combination and rotation is recommended for the control of PRR in China.

32.4.5 Biological Control

Although conventional synthetic agrochemicals have been effective to prevent the outbreak of plant diseases for many years, their use has been significantly reduced in recent years because of increasing public concerns over potential risks to human health, environmental pollution, negative effects on non-target organisms or the development of pest resistance. The concerns support the need to find supplemental or alternative control methods such as biological control (Axel et al. 2012; Benhamou et al. 2012; Putri et al. 2014). Biological control, also called biocontrol, is classically defined as “the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be” (Eilenberg et al. 2001). This definition now should be expanded to include microbial metabolites and other naturally derived compounds (Axel et al. 2012).

In the case of PRR in China, no commercial biocontrol agents are available at present. However, there have been a few evaluations of biocontrol agents for control of *P. sojae*. Several investigators targeted induced resistance as a mechanism to control *P. sojae* using natural agents such as chitosan or Harpin proteins of *Xanthomonas* bacteria (Wang et al. 2008; Shen et al. 2011; Yang et al. 2014). Others explored the effect of different antagonistic microbes (e.g. *Aspergillus effusus*, *Pseudomonas* sp. and *Bacillus* sp.) and plant extracts of e.g. *Albizia lucidior*, *Uncaria sessilifructus* and *Albizia procera* (Fu et al. 2011; Pan et al. 2011; Shen et al. 2011; Han et al. 2012; Liu et al. 2014a, b; Yang et al. 2014). One study showed that mixture of the antagonistic microbe *A. effusus* with chicken-based fermented organic fertilizer can inhibit root rot incidence of soybean in pot experiment while the biomass of soybean plant was promoted by 31–67% (Liu et al. 2014a, b). Amongst the trials conducted so far, biological control agents were mostly screened for their activity *in vitro* and *in situ*, with assays generally being performed under controlled conditions. Recently, different types of biological substances were investigated as biocontrol agents against *P. sojae* in the field (Shen et al. 2011; Yang et al. 2014). Tests revealed that Harpin proteins at 45 mg/L offered the best control effect with the average incidence of PRR at 0.12%, the prevention effect of 75% and average yield being 7% higher than the control (Shen et al. 2011; Yang et al. 2014). The second one was "818" fermentation liquid from the Chinese Academy of Agricultural Sciences with the prevention effect of 58.3% (Shen et al. 2011; Yang et al. 2014). In these field tests, one commercial American biocontrol product, namely NEB (Nutrient Enhancing Balancer) containing arbuscular mycorrhizal, has also been evaluated as potential agent against *P. sojae* (Shen et al. 2011; Yang et al. 2014). It was applied in the form of seed coating agent and offered the prevention effect at 37.5%, showing its promising application in the control of *P. sojae* (Shen et al. 2011; Yang et al. 2014). Additionally, recent studies conducted in Northeast Agricultural University revealed that Borrelidin, one macrolide antibiotics produced by *Streptomycete* sp., showed high and specific anti-fungal activity against *P. sojae* (Gao 2013). The results obtained suggested the possibility of developing Borrelidin as a novel anti-oomycete agent in the future. Nevertheless, continued research and screening of other potential biocontrol agents to control PRR are still required.

32.4.6 Agricultural Control

Once *P. sojae* has become established at a location, it can be extremely difficult to control, especially in wet field conditions. In critical wet periods, *P. sojae* rapidly infects soybean plants by numerous swimming zoospores because a large amount of surface moisture is required for their movement (Erwin and Ribeiro 1996). Most agricultural control strategies aim to limit losses by limiting free water. This is accomplished using a variety of techniques, including planting at well-drained sites, planting on raised beds and the use of carefully controlled (e.g. trickle) irrigation (Schmitthenner 1985). Methods for controlling the diseases also include modifying tillage practices (Workneh et al. 1998) and applying calcium-containing compounds (Sugimoto et al. 2010a). Additionally, crop rotation with non-host plants such as corn or wheat is also recommended (Agrios 2005).

32.5 Conclusion and Outlook

P. sojae, the most devastating soybean oomycete pathogen, has become widespread in many soybean-producing countries (Schmitthenner 1985; Erwin and Ribeiro 1996). In China, it has been listed as a quarantine pathogen since 2007 (http://www.aqsiq.gov.cn/; June 22, 2016). With the increasing amount of soybean traded with different countries, *P. sojae* is found present in shipments with increasing frequency (AQSIQ; http://www.aqsiq.gov.cn/; June 22, 2016). In addition, the increasing incidence of the disease within China has warned us about high risks of domestic spread of the species. Significant economic consequences could result if *P. sojae* was widespread in China. Hence, it is very important to let the society understand how high this risk that we are facing is, and what strategies we are currently taking to control the spread of this pathogen and outbreak of the associated diseases.

Along with the rapid development of Chinese economy, the demand for more rapid, reliable and cost-effective quarantine and treatment technologies is increasing. More studies should be conducted to develop such detection and treatment measures (such as fumigation methods) that can be applied to the imported soybeans at the dock. The pending phase-out of fumigants such as methyl bromide further exacerbates this problem. To date, the most important strategy for control of this pathogen has been the frequent application of traditional fungicides and utilization of resistant cultivars. In times of increasing social pressure to reduce the application of synthesized pesticides on crops, demands for eco-friendly replacements have intensified. Biocontrols using microbes derived from natural sources such as soil, water, plants and other organisms, or using microbial metabolites and other naturally derived compounds are the significant strategies for ecologically sound plant disease management (Agrios 2005). Furthermore, plant pathologists and breeders have recognized the value of partial resistance in soybean, which should be more durable and broader than resistance dependent on a single major gene. Similar benefits could also be achieved by “stacking” two or more *Rps* genes or an *Rps* gene and partial resistance genes in the same background (Sugimoto et al. 2012).

Last but not the least, more research is required to elucidate the mechanisms underlying the pathogenicity of *P. sojae*, the results of which can ultimately help design the effective control strategies for the diseases caused by this soybean oomycete pathogen.

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Chapter 33
Fusarium oxysporum f. sp. *cubense*

Birun Lin and Huifang Shen

Abstract Fusarium wilt of banana, a lethal fungal disease caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc), has become a plague and serious threat in banana-producing regions of the world. Since spread in china, this disease had caused huge conomic losses to the banana industry. In this chapter, we describe the invasion situation and epidemiology of banana disease in china, and discuss the pathogenic mechanism on virulent toxin and virulence genes. Further, we summarize the research progress of molecular detection of pathogens. Finally, we review the research progress on management strategies, including strict quarantine system, breeding and cultivation of disease-resistant varieties, chemical pesticide control, and biological control and cultural control.

Keywords *Fusarium oxysporum* f. sp. *cubense* • Distribution • Pathogenic mechanism • Detection

33.1 Introduction

Fusarium wilt of banana, also known as Panama disease or yellow leaf disease, is caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) (Tuberculariales, Tuberculariaceae). It is a devastating vascular wilt disease. The typical external symptom is leaf chlorosis. Infected leaves collapse on petioles, droop and wilt quickly, turn yellow to brown and dry. Some disease plants crack from the near ground surface leaf sheaths periphery of the pseudostems. The crack expands to the center and up to heart leaves gradually, causing serious damages to leaves. The crack opening is brown with dry rot. Finally, the leaves turn yellow, drooping or not reversed and the plants turn withered slowly (Fig. 33.1). It is a vascular disease with obvious pathological changes in the internal roots and pseudostem. All the vascular bundles in bulbodiums, pseudostems, even ear axes turn yellow or brown. The punctate or linear pathological changes run through

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Fig. 33.1 Characteristics of leaf chlorosis, dry rot and pseudostem crack symptoms of fusarium wilt of Banana (Photo by Birun Lin). *Left*, leaf chlorosis, dry rot. *Right*, pseudostem crack

Fig. 33.2 Characteristics of pseudostem discolour symptoms of fusarium wilt of Banana (Photo by Birun Lin)

themselves to a long strip or block shape. Red brown and black brown conduits in roots extend to the corms and become dry rot finally (Fig. 33.2).

The banana wilt disease was first reported in Australia in 1874 and found in Hawaii, USA in 1904. It caused great losses to the banana production in Panama in 1910. From 1935 to 1939, this disease outbroke in large areas in Panama, Costa Rica, Honduras, Columbia and other countries, which covered more than 90% of the honey quality banana (Gros Michel (AAA) and destroyed about 430,000 hm^2 of banana, causing a great economic loss. Then the disease gradually spread to the banana producing areas of the world through export. In the 1950s, the *Fusarium oxysporum* f. sp. *cubense* race 1 had been present in most regions except South Pacific, the Mediterranean, Malaysia and some other islands. At present, this race is widely distributed in all banana producing areas in Asia, Africa, Australia, South Pacific and tropical America.

33.2 Pathogens of Banana Wilt Disease and Their Spread in China

The pathogen of banana wilt disease has four physiological races. Race 1 is virulent to AAA genomic cultivar 'Gros Michel' and AAB Rasthali, and resisted by Dwarf Cavendish AAA. Race 2 is virulent to 'Bluggoe' of ABB genomic group, some closely related cultivars, and some Jamaica of AAAA group in Honduras, Salvatore, Puerto brother, the Republic of Dominica and the Virgin Islands in American, but it is resisted by cultivar 'Gros Michel' AAA. Race 3 is only virulent to *Musaceae Heliconia* (HeliconiaL.) plants under natural conditions and infects cultivar 'Gros Michel'(AAA) and Musa balbisiana (BB) weakly. Race 4 is virulent to cultivar 'Gros Michel', 'Dwarf Cavendish', 'Musa balbisiana' (BB), 'Bluggoe'. It infects AAA genomic cultivar 'Cavendish' seriously, which is resistant to Race 1.

In China, Races 1 and 4 are the main physiological races; Race 1 mainly damages *Musa* ABB, and Race 4 damages *Musa* ABB and *Musa* AAA Cavendish. Race 1 was found in Guangdong province in 1970s and large areas of Musa ABB and AAB were infected. The incidence area was only 1.4 hm^2 in 1995, while largely increased to 20,000 hm^2 in 2003. In 2005, due to the serious damage from fusarium wilt, banana in some parts of Naozhou Island, Zhanjiang were replaced by other fruit trees. This race also ever caused serious damages in some areas of the Pearl River Delta in Guangdong. It has spread to Hainan, Fujian, Guangxi provinces later.

Race 4 was first reported in Taiwan in 1967 and was epidemic in 1970s. In Taiwan, damages of race 4 were extremely serious, and the banana planting area tremendously reduced from the peak 50,000 to the recent 5000 hm^2, and half of them are still affected by fusarium wilt today. In 1996, the suspected Race 4 infecting banana was found in Panyu of Guangdong, and soon after this disease spread rapidly in China. In 2001, the pathogen was identified as Race 4 coming from Taiwan. Race 4 caused fusarium wilt on Baxijiao (*Musa* AAA Cavendish cv. Baxi) in Phoenix Town, Sanya, Hainan province in December 2001. Now, this disease has spread to major banana plant areas in Guangdong and brought huge economic losses in banana production.

So far, fusarium wilt of banana has been found in Guangzhou, Zhuhai, Shantou, Huizhou, Dongguan, Zhongshan, Jiangmen, Zhanjiang and Maoming in Guangdong Province; Nanning, Qinzhou and Baise in Guangxi Province; Zhangzhou in Fujian Province; Haikou and Wenchang in Hainan Province, Honghezhou, Xishuangbanna, Puer and Lincang in Yunnan Province. But in recent years, as the disease has occurred sporadically in large areas, the fungal pathogen still has high risk of spreading to a broader range.

Seedlings, water source, soil and fertilization are four main elements for epidemic fusarium wilt in China. Infected seedlings and irrigating water from fusarium wilt disease affected area are the two key factors for the pathogen spread in non-infected areas. In addition, the content of organic matter, aeration, water retention and pH are also associated with fusarium wilt disease. It has been reported that the disease incidence rate and the spread rate would reduce significantly when organic fertilizer instead of urea and other chemical fertilizer was used.

33.3 The Pathogenic Mechanism of Fusarium Wilt of Banana

33.3.1 Virulent Toxin

In the pathogenesis of fusarium wilt of banana, most studies focused on the toxin. The pathogen produces some substances during the interaction with host plants, such as hormone, enzymes and toxins, which cause serious harms to the host. Toxins play an important role in the pathogenesis of *Fusarium oxysporum*, especially after invading its hosts. By combining with some proteins in cell plasma membrane, toxins damages the membranes' structure and function, resulting in membrane permeability change, electrolyte leakage, conductance value increase, and the withering of the whole plant.

Toxins produced by *Fusarium oxysporum* do obvious harm to host plants. Xu et al. (2004a, b) showed that parenchyma and meristematic cells of banana vascular bundle could produce jelly and brown pathological reaction after treated with crude toxin of *F. oxysporum*, and the pathological changes did not differ among races. Fusaric acid has been proved to be the most important factor for Races 1 and 4, causing fusarium wilt by means of root membrane destruction, metabolic disturbance and host root defense function loss.

In addition to the fusaric acid, there are also other components in toxins of *F. oxysporum*. Li et al. (2010a) reported that toxin of Race 4 contained seven components and fusaric acid was the most important. Non host selective toxin, found in crude toxins, was another virulence factor for fusarium wilt. Li et al. (2011a) observed the leaf ultrastructure of Baxijiao (*Musa* AAA Cavendish cv.Baxi) seedlings treated with crude toxins and pure fusaric acid of Race 4. The cells were destroyed as a result of cell plasmolysis, the number of chloroplast and internal starch granules decreased, osmiophilic granules appearance, chloroplast lamellaes decomposition, mitochondria deformation and inclusion outflowed. (Li et al. 2011a).

Li et al. (2010b) reported that Both Races 1 and 4 could produce fusaric acid, but Race 1 produced more than Race 4. In addition, beauvericin was another virulence toxin produced by race 4, which caused wilt rot of banana pesudostem (Li et al. 2011b).

The toxins provide a new idea for banana fusarium wilt resistance ability evaluation. Xu et al. (2008) gained more than 100 regenerated plants by means of toxin as selection pressure for in vitro resistance screening. As an effective method of rapid distinction of banana resistant ability, Yang et al. (2014) measured leaf damage area after banana middle leaves treated with toxins for 72 h. Li et al. (2015) has screened and gained some resistant varieties by the technology of physical and chemical mutagenesis, toxin screening of fusarium wilt resistance breeding and bud mutation breeding.

33.3.2 Isolation of Virulence Genes

Some special genes involve in pathogenic processes of *Fusarium oxysporum*. The *fgal* gene is required for spore germination. Deletion of this gene will reduce expression levels of cAMP in pathogen cells and weaken the pathogenicity. Li et al. (2011c) cloned *fgal* gene from Races1 and 4. The nucleotide sequences from these two races shared 99% identities to *fgal* from *F. oxysporum* f. sp. *lycopersici*. The Race1-*fgal* gene is conservative. However, the alternative splicing exists in Race 4-*fgal* gene, which may be one reason for high virulence of Race 4. The *fmkl gene* deletion causes many defects associated with the function of toxicity, such as root attachment, penetration and invasive growth, pectate lyase secretion, hyphal growth and fusion. Yang et al. (2011) cloned *focMK* gene from Races 1 and 4, and found only one different nucleotide between them, which share high identity to *focMK* from *F. oxysporum* f. sp. *lycopersici*. The *Ste12* gene, downstream of *fmkL* gene, has been cloned from Races 1 and 4 and found 7 nucleotides but only one amino acid difference (Zhou et al. 2011).

Transcription factor regulates physiological processes by regulating the expression of multiple genes. Their mutation may prove some new ways of regulation. There is obvious difference among gene sequence and amino acid sequence of Race 1-*PacC*, Race 4-*PacC* and other *PacC genes*. This indicated that the *pacC* gene is less conservative (Li et al. 2010c). Potential function of conservative *PacC* will be further investigated in future work. *Fusarium oxysporum* f. sp. *cubense* can secrete cell wall degrading enzymes to decompose pectin, cellulose and hemicellulose of host cell wall for successful invasion. Cell wall degrading enzymes play an important role in the pathogenesis of banana fusarium wilt. One glucosidase gene, *Foc*-3076 was cloned by Li et al. (2010c). Glucosidase is a kind of cellulase, which degraded cellulose in plant host cell wall to damage cell wall structure. *Polygalacturonase* (PG) can degrade homologous polygalacturonase region in the plant cell wall to cause tissue maceration and protoplast death. *pgx4* has been cloned from Races 1 and 4 (Yang et al. 2009), this gene has a difference of 25 nucleotides and three amino acids between the two races. Moreover, an exopolygalacturonase has been isolated and characterized for the two races (Dong and Wang 2011). *FoABC1* gene, may code a kind of ABC transport protein responsible for pumping toxins, was cloned from Race 4 (Li et al. 2006). The *esyn1* gene, cloned from Race 4, is one most important gene for fusanin and beauvericin biosynthesis (Chen et al. 2011).

T-DNA insertion mutant library of banana fusarium wilt Race 1 and Race 4 has established by Biology Institute, China Academy of Tropical Agriculture and three virulence related genes were identified. Among them, *mpfo1* gene (from Race 1) codes reticuloendothelial fibroin which controls the polar cell growth. Insertional inactivation gene of mutant *Focr1-N2-328* is related with strains carbon source utilization, acid production regulation and hyphal penetration ability. Insertional inactivation gene of mutant *Focr4-1701*, coding Helicase, is related with hyphal penetration ability, pectinase activity, ability of toxin production and maltose utilization.

33.4 Molecular Detection of Pathogens

Banana fusarium wilt spreads by infected soil and seedlings. And rapid detection is of important significance to prevent the spread of fusarium wilt disease. Several studies have developed PCR-based methods for detecting race 4. For example, Lin et al. (2010) designed a 404-bp PCR primer that specifically targets Race 4 and used the SYBR GREEN fluorescent dye in real-time PCR for identification. Protocols based on loop-mediated isothermal amplification (LAMP) have been developed to detect Foc race 4 in soils and infected banana plant tissues (Li et al., 2013; Zhang et al. 2013b). As such LAMP methods were simple, rapid, sensitive, and specific, they can be used for the detection and monitoring of Foc TR4 in fields.

Molecular methods for identifying and distinguishing different banana fusarium wilt races have also been developed. The internal transcription spacer sequence of banana fusarium wilt race 4 is different from sequences of Race 1 and other ITS of *Fusarium oxysporum* strains at 367 bp and 386 bp. Based on such difference, Wang et al. (2007) designed three primers that targeted ITS region using endpoint PCR, and Lv et al. (2009) developed invented dual PCR on the basis of ITS-SCAR to identify Race 1 and Race 4. Using the technique of the RAPD molecular markers, Lin et al. (2009) labeled primer pair Foc-1/Foc-2 designed from 242 bp specific sequence amplified with random primer OP-A02. By combined real-time PCR, the higher sensitivity, more stable, SCAR molecular marker primer pair FocSc-1/FocSc-2 was designed. Chinese scientists have also established the banana fusarium wilt rapid detection technology based on sequence characterized amplified region (SCAR) markers. For example, Liu et al. (2006) has achieved one SACR marker for Race 1, two markers for Race 4, and one marker for both Race 1 and 4; application of these markers in nine field-isolated pathogens showed that they could be reliably used for identification of Race 1 and Race 4. Liao et al. (2009) also found one SACR marker for Race 4. Li et al. (2012) developed a triplex PCR-based system that could rapidly detect and distinguish these two races.

Most recently, Yang et al. (2015) established a highly efficient, single-tube duplex real-time fluorescent PCR detection system for the identification and differentiation of Race 1 and Race 4. Using this system, the two races can be detected simultaneously, and the detection can be completed within 90 min (not including DNA extraction) with a sensitivity one to two magnitude orders higher than conventional PCR. Therefore, this method can be utilized as an accurate and sensitive method for the monitoring of the two races.

33.5 Technology of Prevention and Control

33.5.1 Strict Quarantine System

Banana seedlings are the main way of long-distance spread of Banana fusarium wilt, therefore, it must be from non infected areas. The cultivation and management measures will be strengthened, for example, diseased plants removal and infected area isolation.

33.5.2 Breeding and Cultivation of Disease – Resistant Varieties

Since the beginning of this century, several disease-resistant cultivars have been introduced to mainland China, such as "Xinbeijiao" (*Musa* AAA, Cavendish cv Formosana, also named "Baodaojiao", introduced in 2002 from Taiwan province), and "Kangku 5" (introduced in 2003 from International Network for the Improvement of Banana and Plantain, INIBAP). A number of resistant cultivars had also been bred by Chinese institutions, including Kangku 1, Haigong, Fenza 1, Nongke 1,Yuke 1, and Zhongshan kanghuang 1 (Liu et al. 2012). Some of them have been widely cultivated.

Chen et al. (2013) obtained five wilt-resistance Brazil banana (Musa spp., AAA) lines, using the method of combining micro-cross section cultural system and ethyl methanesulphonate-induced *in vitro* mutagenesis. Most recently, by screening eight genotypes of wild banana relatives, Li et al. (2015) identified two species (*Musa basjoo* and *M. itinerans*) that are of higher levels of resistance to Foc tropical race 4 and can be used in banana breeding programs for generating new resistant cultivars.

33.5.3 Chemical Pesticide Control

Currently, there is no effective chemicals for fusarium wilt control. In view of the ineffective control caused by serious chemical pesticide loss, high water absorbent resin and water retaining agent was mixed into pesticides to improve the application effect significantly. The disease incidence rate decreases obviously in the early of chemical pesticide application. It has been proved that treating soil of banana seedlings planting with lime nitrogen could reduce the incidence and disease index of fusarium wilt disease effectively.

It is difficult to kill the fungal pathogen of banana fusarium wilt due to concentration in the vascular bundle. This disease is difficult to control effectively by the traditional methods of constant exposure to the sun, quicklime sprinkle, spraying of glyphosate, centralized burning or deep buried. Liu et al. (2015) invented KF (main ingredients: prochloraz, carbendazim and glyphosate), established technology of 'drilling for pesticide application' to control Banana fusarium wilt. Once diseased plant was found, he advocated 'timely removal' to avoid the formation of disease center.

33.5.4 Biological Control

In the past decade, 删除 several Recently, researchers focus on biological control and obtain some results by a lot of related biocontrol experiment.

Fungus: *Trichoderma* spp. widely exist in nature. Due to the characteristics of strong vitality, fast reproduction, strong competition ability of living space and nutrients, they can suppress growth of *Fusarium oxysporum*. A number of isolates of *Paecilomyces lilacinus* and *Trichoderma* spp. with strong inhibitory effect have been obtained (Mo et al. 2012; Wang et al. 2013).

Bacterium: *Pseudomonas* spp. (e.g., *P. fluorescence*), which colonize roots of banana and are capable of inducing systemic resistance to fusarium wilt, have been isolated. Some of them were tested to be able to substantially suppress *Fusarium oxysporum* f. sp. *cubense*, by inhibiting hyphal growth and expansion of the pathogen (Huang et al., 2006). They have the advantages of adapting well to root environments and proliferating rapidly. However, they are unable to kill the pathogen. Some *Bacillus* isolates capable of strongly inhibiting Race 4 have also been obtained, such as *Bacillus licheniformis* (Sun and Wang 2009; Yang et al. 2010) and *Bacillus subtilis* (Sun et al. 2008). In addition, some endophytic nitrogen-fixing bacteria can also inhibit *Fusarium oxysporum* f. sp. *cubense* proliferation.

Actinomycetes: *Streptomyces olivochromogenes* strain DA07408 with activity against fusarium wilt was isolated from soil (Qiu et al. 2009). In addition, *Streptomyces* strain HW1, isolated from hot spring in Hainan, showed high inhibition effect on Race 4.

Now, two microbial control strains have been registered for commercial use, which are *P. lilacinus* and *B. subtilis*. Wang et al. (2013) suggested combining the use of *P. lilacinus* with intercropping of sweet potato to increase the overall control efficiency against fusarium wilt. This approach can promote banana growth and increase abundance of rhizosphere microorganisms.

33.5.5 Cultural Control

Crude extract from several plants have been demonstrated to be able to inhibit Foc TR4, such as Chinese leek *Allium tuberosum* (Huang et al. 2011, 2012; Yang et al. 2012, 2014; Mu et al. 2014), garlic (Mu et al. 2014), green Chinese onion *Allium fistulosum* (Mu et al. 2014; Yang et al. 2014), sunflower *Helianthus annuus* (Pu et al.

2015), Leafy Lettuce *Lactuca sativa* (Pu et al. 2015). For example, the crude extract of Chinese leek could significantly inhibited mycelia growth and conidium germination of Race 4, and induced cell death by inducing oxidative bursts, mitochondrial impairment, and plasma membrane depolarization (Zuo et al. 2015). Moreover, this plant can regulate soil microbial community structure, which makes the soil more resistant to the pathogen (Huang et al. 2011; Yang et al. 2012). These findings suggest that, planting some crops, by rotation with the banana, is a choice for the control of the pathogen. For example, intercropping and rotating banana with Chinese leek and pineapple appeared to be an ideal choice to be used in environmental friendly control programs of fusarium wilt of banana (Huang et al. 2012; Zhang et al. 2013a; Wang et al. 2015).

33.6 Conclusions and Future Directions

Fusarium wilt of banana is regarded as one of the most devastating diseases of banana, and becoming a major constrain to banana production in China. To date, Chinese researchers have made significant progress in molecular detection of pathogens, the pathogenic mechanism and management of this disease. These results can provide technical support for effectively inhibiting the proliferation and invasions of pathogen of banana wilt disease, and controlling the disease.

Although considerable progress has been made in the pathogenic mechanism of fusarium wilt of banana, there are still many limitations and challenges. For instance, in terms of composition and mechanism of pathogen toxins, not a sufficient number of pathogenic toxin types have been isolated and identified.

Development of resistant cultivars is considered as the most economical and environmental friendly method to control the disease. Although many breeding programs have successfully developed now, there are few resistance genes and resistant varieties. Unfortunately, it cannot cover the production, and practically no cultivar resistance to tropical race 4. Fortunately, given that biotechnology breeding has become the focus of modern resistance breeding, there is a critical need to be concentrated to identify resistance genes with genomics and proteomics, and reveal the mechanism of their complementary defense molecular components in the host. Only in this way can we create the conditions for molecular breeding, and become one of most potential research field in banana.

Most of chemical pesticides can suppress the pathogen obviously in the lab but can not control the disease in the field. In this regard, it is extremely urgent to develop new types of high efficiency pesticides. Significant biological control of fusarium wilt of banana was achieved in some of these studies in China, but there are still many problems. For instance, the mechanisms of biological control products were undefined that cause the differences between inhibitory effect in lab and control effect in the field. At the same time, climate and soil are closely associated with both disease epidemic and effect of biological control, which should be analyzed in the future. It is conceivable that control efficiency of biocontrol agents are affected by cultural system and practices, such as intercropping banana with other crops, which should be further determined.

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Part IV
Perspectives and Future Research Directions

Chapter 34
Research and Management of Biological Invasions in China: Future Perspectives

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Abstract In the past decades, China has made great achievements in both fundamental research and applied management of biological invasions. Meanwhile, upon the increasing of international trade and global changes as well as the initiating of new opening-up strategy, China is facing an even more serious threat from effects of globalization. Here we propose future perspectives on both studying and managing biological invasions in China. Based on the current research progress and future trends of biological invasions, China should strengthen studies in the following areas: (i) carrying out extensive surveys on current status of invasive alien species (IAS); (ii) deeply investigating mechanisms of invasion success and species invasiveness; (iii) evaluating the invasibility of various ecosystems; (iv) developing methods for rapid detection and sustainable control; (v) strengthening governments' roles; and (vi) promoting international cooperation. We emphasize the necessity of directing more efforts towards research areas that were poorly studied before, such as ecosystem invasibility, and the invasions in nature reserves and grasslands. In addition, strengthening governments' roles would be also crucial to build an effective IAS managing framework in China.

Keywords Global changes • Sustainable management • Invasiveness • Invasibility • Governmental roles • Ecosystems • "One Belt and One road"

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34.1 Introduction

China is among the countries seriously affected by biological invasions, suffering huge losses in economy, ecology and human welfares (Ding et al. 2008; Wan et al. 2009). To resolve this issue, China has made great efforts on both fundamental research and applied management. In the last decade, particularly, great progress has been made in the field of biological invasions and our ability to deal with invasive alien species (IAS) got improved significantly (Wan et al. 2009).

However, similarly to many countries in the world, China is facing an even more serious threat from biological invasions. It has been evidenced that the number of IAS introduced into China reaches 614 by the end of 2015, of which 50 are among the world's 100 worst IAS. Moreover, novel IAS have invaded China at an unprecedented high rate. For example, as many as 55 IAS were firstly recorded during 2006–2015, with an increasing rate 2.5 times higher than before (data from Center for Management of Invasive Alien Species, China).

It should also be noted that biological invasions in China are facilitated by more factors as compared with other countries. In addition to the well known global factors, *i.e.*, increased international trade, human activities and global climate change (Vitousek et al. 1996; Leprieur et al. 2008; Bradley et al. 2010; Seebens et al. 2015), there are also a few more specific to China: large national territory, long land border and coastline, diverse habitats, rapid expansion of transport networks, and initiation of new opening-up strategies (*e.g.*, establishing free-trade zones and "One Belt and One Road"). All these factors can create more opportunities for alien species to be introduced into China and/or to be facilitated to spread over a broader range in the country.

Thus, China will still be challenged by biological invasions and potentially suffer more severe damages in coming decades. In this chapter, we discuss and identify the research gaps in the field of biological invasions in China and future studies to be conducted to fill these gaps in both fundamental research and applied management.

34.2 Extensive Surveys on Current IAS Status

Based on historical records, extensive national surveys (performed during 2001–2003 and 2008–2010), and recent data collection, we have a relatively comprehensive background on invasive species in China (Ding et al. 2011; Xu et al. 2012; CMIAS, Database of IAS in China). However, owing to the difficulty in collecting some species that are in low abundance or reside in out-of-reach habitats (*e.g.*, below part of deep water bodies, and crown of tall trees), the number of IAS in China may have been underestimated. Given that IAS are continually increasing, and 6 years have passed since last nation-wide survey, to launch a new comprehensive survey is urgent in coming years to get a thorough list. More efforts should be given to the ecosystems that had been overlooked, such as nature reserves, deserts oasis, grasslands and aquatic habitats. Due to the rapid increase of human activities (in particular tourism), these ecosystems will have much higher risk to be invaded by alien species and the current IAS status should be investigated as soon as possible.

34.3 Investigating Invasion Process and Species Invasiveness

After introduced to nonnative range, alien organisms experience several stages before becoming invasive and causing significant effects. Such stages include establishment, lag period, spread and outbreaks (Sakai et al. 2001). Deep investigation of the whole process is extremely essential for risk assessment, early warning and prevention. Unfortunately, among well studied IAS in China, most are terrestrial ones in agricultural and forest ecosystems and only a few are reported in other types of ecosystems.

The ability of alien plant and arthropod species to invade new range (invasiveness) has been extensively studied so far and numerous valuable findings were obtained (Wan et al. 2005; also see Chap. 1). However, lots of previous studies were often not systematic, and thus did not result in common finds. Moreover, only a few studies focused on genetic mechanisms of invasiveness and adaptive evolution. These two aspects are closely related with invasion potentials, unfortunately knowledge in this field is limited to a few plants. In the future, physiological and genetic basis and rapid evolution should be deeply investigated, particularly on traits that play crucial roles responsible for invasiveness. The IAS newly introduced into China should be studied urgently on their invasive capacity.

34.4 Evaluating Invasibility of Ecosystems

Invasibility of ecosystems is attracting much research attention mainly owing to its significant relationship with invasion outcomes. Invasibility can be affected by a number of factors, such as biodiversity, disturbance regime and resource availability. As compared with species invasiveness, invasibility is poorly understood in China.

In particularly, the contribution of human-induced disturbance to invisibility should be further studied. In China, the pattern of agricultural land use has changed tremendously since 1990s due to rapid urbanization and industrialization, which has in turn changed biodiversity and landscape in many regions (Lu et al. 2011; Chou et al. 2015; Zhao et al. 2015). Along with the increase of nitrogen deposition in terrestrial and aquatic ecosystems, the N allocation pattern in plants and soils has been changing (Liu et al. 2013). As tourism develops rapidly, human disturbance to grassland, nature reserves, desert/oases and grasslands has been intensified (Zhang et al. 2012; Jia et al. 2014). Heavy grazing in some desert steppe rangelands has altered local plant communities (Deng et al. 2014). All these factors can potentially affect invasibility of ecosystems.

We suggest taking the following points into account when studying invasibility: (i) examining interactions of multiple factors in the ecosystem and their joint and respective effects on invasibility; (ii) investigating the underlying mechanisms in changes of invasibility; (iii) monitoring the temporal changes of invasibility over time; (iv) quantifying invasibility to indices that could be adopted for effective management (Guo et al. 2015).

34.5 Developing Detection Methods

In the past decade, DNA-based detection approaches have been developed for a great number of IAS in China (Wan et al. 2011; Xiong et al. 2016). Still, more reliable, easy-to-operate detection tools in particular the DNA-based ones are to be developed. Such tools will be very helpful for inspection and quarantine at entry-exit ports and quarantine stations, where there are urgent needs to identify/distinguish tiny or morphologically similar organisms rapidly and accurately. Remote rapid real-time detection platforms are to be established across the country.

34.6 Developing Sustainable Control Methods

To manage the IAS that have already distributed widely with large populations, developing low-cost, environmental friendly and sustainable control methods is the first priority. For invasive terrestrial animals, such methods mainly include the use of biological control agents, traps, repellants and sterile techniques, while for invasive terrestrial plants, using biocontrol agents and replacing with appropriate native plants are much desirable. Growing resistant crops is the most promising approach to reduce damage from arthropod herbivores and pathogens. In China, some of these methods have been widely adopted in agricultural and forest ecosystems (Yang et al. 2014; Wan and Yang 2016).

34.6.1 Biological Control

Biological control is applicable for most of terrestrial ecosystems, such as agricultural lands, forests, nature reserves and grasslands. In China, this control strategy has been successfully employed in farms, greenhouses and forests (Wan et al. 2008). In the other types of ecosystems, however, it is much less explored. We provide the following suggestions to improve the application of biological control to IAS in the future.

(i) Developing mass-rearing technique of biocontrol agents. This work is urgent for the agents are used to control IAS that are highly invasive [*e.g.*, common ragweed *Ambrosia artemisiifolia* L., sweet potato whitefly *Bemisia tabaci* Gennadius, and oriental fruit fly *Bactrocera dorsalis* (Hendel)], secretive and thus difficult to be controlled by other methods [*e.g.*, coconut leaf beetle *Brontispa longissima* (Gestro)], or the ones dispersing in residential areas where other control methods are not applicable [*e.g.*, fall webworm *Hyphantria cunea* (Drury)];
(ii) Increasing the biocontrol agents' tolerance to abiotic stresses, such as low/high temperature and desiccation met during storage, transportation and after release (Zhao et al. 2012, 2014);
(iii) Seeking complementary methods to enhance control efficacy of the released agents, such as the methods developed for the parasitoid *Encarsia formosa* Gaha released to greenhouses in northern China (Zhang et al. 2010); and
(iv) Improving chemical use strategy to reduce negative impacts on biocontrol agents.

34.6.2 Replacement Control

Replacement using valuable native plant species is a sustainable method for controlling some invasive plants (Li et al. 2015a). Once the replacement succeeds, the target invasive plants have low possibility to re-establish at a large scale. This method is rather practical in complex landscapes, such as tidal-flat areas and steep slopes where it is difficult to employ other control measures. In China, successful cases have been reported in the control of *A. artemisiifolia*, crofton weed *Ageratina adenophora* (Spreng.) King & Rob., and *Spartina alterniflora* Loisel (Guan et al. 1995; Zhong et al. 2007; Ou et al. 2012; Feng et al. 2014).

Studies on replacement control normally need to last for a long period of time. Future work would include: (a) screening more promising plants that have high replacing potential and high economic value; and (b) investigating the ecological outcomes generated from the use of this method. Special attention should be paid when using exotic plants for replacement purposes (*e.g.*, Zhou et al. 2015), because themselves may potentially become invasive.

34.6.3 Resistant Crop Cultivars

A great number of plant germplasms have been found in China which are resistant to specific pathogens, such as *Fusarium oxysporum* f. sp. *cubense* Schlechtend (Smith) Snyder & Hansen Race 4 (Li et al. 2015b) and *Phytophthora sojae* Kaufmann & Gerdemann (Chaps. 32 and 33). Yet, no resistant cultivars are available for commercial use.

34.6.4 Sterile Insect Techniques

The sterile insect technique (SIT) has been successfully used for the control of invasive fruit flies (Ant et al. 2012). It is accomplished by releasing males that have been previously sexually sterilized through radiation or genetically transformed with a lethal gene(s). In China, now this technique has been also in development for the control of oriental fruit fly, Chinese citrus fly *Bactrocera minax* (Enderlein), and codling moth *Cydia pomonella* (L.) (Ji et al. 2013; Dong et al. 2014; Zhou et al. 2016).

34.7 Strengthening Governments' Role

For laws and regulations so far enacted in China, provisions have been included on target management of intentionally or unintentionally introduced alien species (Zhao 2009). However, for the species to be intentionally introduced, there is lacking provisions that the invasion risk of the species must be previously evaluated before approved for its introduction (Song 2012; Xu et al. 2014). The laws and regulations should be enforced more strictly.

Different government agencies relevant to IAS management are expected to act with more coordination. In China, many agencies are in charge of IAS, including Ministry of Agriculture (MOA), State Forestry Administration (SFA), Ministry of Environmental Protection (MEP), and General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ). Each of these agencies has clear responsibility. However, they act independently, not fully sharing IAS information with each other. Thus some of their activities are overlapped when dealing with IAS issues. Hence, a national guideline is needed under which the efforts from all involved agencies can be integrated organically, thereby generating an optimal IAS management strategy. Such coordination is relatively important when the targeted invasive species occur in more than one ecosystem and need actions of various agencies.

It has been frequently suggested in China that the public should increase their awareness on biological invasions. Unfortunately, a proportion of the public is often found to carry, maintain or release prohibited organisms or living materials without consciousness. We suggest governments to design a long-term mandatory education plan for the public. When implementing education programs, diverse tools/approaches can be used to better outcomes, such as TV, radio, smart phone, internet, lecture, scientific booklet, leaflet, poster, field travel, scene plays, *etc*.

The financial support from governments is to be increased. Since the beginning of this century, Chinese governments have launched a number of big research projects to promote IAS research and management. More recently, biological invasion was listed as one of major topics in the national research program "Key technology research and development of biological safety" (2016–2020). However, as there are so many biological invasion issues in China to be resolved, the funding is apparently not sufficient. Chinese governments are expected to set up a long-term budget specifically to research and management of IAS.

34.8 Promoting International Cooperation

A close international cooperation is very important for China to effectively manage IAS. Upon the increase of trade and exchange with other countries, more IAS may be introduced into China. To resolve the upcoming biological invasion issues, it is urgent for China to consult with the countries neighboring free-trade zones and those connected to "One Belt and One Road". Partner countries are expected to design joint plans for IAS risk assessment, early warning, quarantine, monitoring, and prevention.

34.9 Conclusions

In China, biological invasions have arisen as a major threat to agriculture, biodiversity and other human welfares. In this context, China will have to exert considerable efforts to research and management of invasive species in coming decades. Species invasiveness and ecosystem invasibility will still be the major areas in future research. In particular, the poorly studied invasibility should be extensively investigated. More studies should be designed to learn how invasibility changes under global and local changes, so that measures can be found and used to increase ecosystem's resistance to IAS. As compared with agricultural and forest ecosystems, other ecosystems (water bodies, nature reserves, desert oases and grasslands) were much less studied before and should be given more research efforts in the future.

Rapid detection has to be developed for newly and/or potentially introduced IAS. To realize sustainable management of IAS, more low-cost, environment-friendly control methods are to be developed. Chinese governments are expected to play more roles in legislation, coordination and education of the public to build a more effective IAS management framework.

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