Chapter 8
Non-chemical Seed Treatment in the Control of Seed-Borne Pathogens

Eckhard Koch and Steven J. Roberts

Abstract Non-chemical seed treatments include physical treatments, microbial treatments and treatments with other agents of natural origin like plant powders or extracts. Physical treatments with hot water, aerated steam, or dry heat have successfully been applied to a range of crops against a range of target pathogens and are in commercial use primarily for vegetable seeds. They can be very effective but need to be optimised on a per seed lot basis.

Microbial seed treatments may control not only seed-borne pathogens but also provide some protection against pathogenic soil-borne inoculum. However, research on the use of micro-organisms as seed treatments has been limited, and there are only a few examples of commercial use. The latter is also true for botanical seed treatments, despite many reports of bactericidal and fungicidal effects of compounds from plants. The reason may be a lack of research on the one hand but mainly commercial constraints like development and registration costs in relation to market size.

The current chapter gives an overview of approaches that have been taken to utilize the above-described non-chemical methods for control of important seed-borne pathogens of vegetables and small grain cereals. The examples treated include bacterial (black rot of brassicas, pea bacterial blight, bacterial blotch of cucurbits, black chaff of cereals), fungal (*Alternaria* diseases of carrot, black leg of brassicas, common bunt of wheat, *Fusarium* seedling diseases of small grain cereals, the loose smuts of barley and wheat, fungal diseases of rice and sorghum) and important viral diseases.

Keywords Biological control • Natural products • Organic farming • Efficacy • Bacteria • Fungi • Viruses

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1 Introduction

Seed treatment is known to have been practised since the mid seventeenth century. Hot water treatment was first reported in the 1880s, but for most of the history of modern agriculture, the majority of seed treatments have been chemicals targeting fungal pathogens, with physical treatments used for bacteria and viruses where there were no chemical options. The growing public concern about environmental risks associated with the use of agrochemicals, the political will to reduce pesticide use (as in the EU), and the development of the organic movement that prohibits the use of synthetic seed treatments all contribute to the current resurgence of interest in non-chemical seed treatments. To many people "non-chemical" means something coming from nature that is safe for humans and the environment. In the context of plant protection and IPM the term generally encompasses precautionary measures, the utilisation of natural mechanisms of control as well as treatments with control agents that are not chemically synthesised. For the purposes of this chapter we follow the latter concept and define non-chemical seed treatments as including all those treatments which are not considered as conventional synthetic pesticides. Thus we will consider physical treatments, microbial treatments, and treatments with natural products.

The main requirements for an "ideal" seed treatment are identical for chemical and non-chemical seed treatment methods. Both should in the first place reduce the numbers or transmission of the target pathogen(s) from the seed to the shoot to acceptable levels. They should further: not reduce germination or vigour; not reduce storability; have low toxicity to humans/animals; not harm the environment. For any kind of seed treatment the location of the pathogen on the seed has significant implications for the likelihood of achieving satisfactory levels of control. Pathogen inoculum may be superficial or internal. Superficial inoculum is located on the surface of the seed/fruit (most bacteria and many fungi) and is easier to eradicate. Internal inoculum may be located in the testa/pericarp (many fungi, some viruses), in the endosperm/cotyledons (a few fungi), or in the embryonic axis (viruses, certain smuts). Chemical seed treatments may contain different active ingredients which may also protect against soil-borne pathogens or, in the case of systemic compounds, provide transient protection against air-borne inoculum, e.g. from powdery mildew. Non-chemical seed treatments have activity primarily against pathogens on or in seeds, some may in addition provide a certain level of protection against soil-borne pathogens.

There is a lack of consistency amongst the various studies in the way results are interpreted and summarized. It is important to consider that the efficacy of seed treatments can only be determined in terms of the seed test or trials used to assess them. Thus it is vital to pay attention to the details of the tests or trials used to assess treatments, particularly the numbers of seeds examined or sown. We have therefore attempted to introduce some consistency by careful re-examination of results in terms of the detection limit or tolerance standard (Roberts et al. 1993) of the test applied. Where possible, we indicate efficacy in terms of the percentage reduction in seed infestation levels or pathogen numbers achieved by the treatment.

In this chapter we summarise the principle non-chemical seed treatment methods, give an overview of approaches that have been taken and also include information on non-chemical seed treatment products and technologies that are already in commercial use. In view of the extensive literature and the large number of seed-borne pathogens we will give specific examples for some important crops. We will preferably summarize results from field- and greenhouse experiments, where available, and avoid laboratory results e.g. on in-vitro testing aimed at characterising the fungicidal or bactericidal potential of putative control agents. The goal is to provide an overview of the current status of non-chemical seed treatments. The information should allow the identification not only of bottlenecks but also their future potential and prospects.

2 Principal Methods of Non-chemical Seed Treatment

2.1 Physical Treatments

Physical treatments have a number of advantages over other treatments: in most countries they do not require registration or approval; they have a wide spectrum of activity; they do not leave any toxic or polluting residues. The latter means that treated seed can also be used for other purposes, e.g. animal feed. The main disadvantages are: the need for optimisation on a per seed lot basis; possible high energy and capital costs; no effects on soil-borne pathogens.

2.1.1 Heat Treatments

Heat treatment or thermotherapy is based on the principal that pathogens are often killed or inhibited at temperatures that are not, or only slightly, deleterious to the seed (Baker 1962). Due to differences in thermal exchange efficiencies, the temperature or time required for successful treatment increases in the order: hot water, aerated steam, dry air (Baker 1972).

Before effective chemical seed treatments became available in the second half of the twentieth century, hot water treatments were widely used for the sanitization of vegetable and cereal seeds. The main disadvantage is the need for post treatment drying. Hot water treatments on cereals (e.g. for loose smut control) and vegetables (e.g. for control of *Phoma lingam* or *Xanthomonas campestris* on crucifer seeds) were commonly performed at temperatures between 50 °C and 55 °C and with durations from 3 to 25 min (Walker 1948; Baker 1962; Gratwick and Southey 1986). A compilation of hot water seed treatment conditions for different vegetables is available on the internet (McGrath 2013). For control of specific cereal pathogens, certain variants were in use, like a discontinuous hot water treatment or a warm water treatment (Jahn 2008). Hot water treatments are still important for the treatment of various kinds of vegetative plant propagation material. Examples of commercial use include the eradication of the bacterium *Leifsonia xyli* subsp. *xyli*, the causal agent of ratoon stunting disease of sugarcane from seed canes (Johnson and Tyagi 2010), the management of nematodes transmitted by suckers of banana and plantain (Coyne et al. 2010) and nematodes in narcissus bulbs (Qiu et al. 1993). For general descriptions of the treatment of vegetative plant propagation material by physical methods the reader is referred to the overviews by Baker (1962), Gratwick and Southey (1986) and Grondeau and Samson (1994).

Compared to treatment in water, the main advantages of seed treatment with aerated steam are a more accurate temperature control, usually less impairment of seed germination and that the seeds are left much dryer. On the other hand, there has been only limited success against bacterial diseases (Baker 1972; Navaratnam et al. 1980). In Sweden, a technology has been developed that is based on high precision control of treatment temperature and humidity and application of the aerated steam in fluidized beds (Thermoseed®) (Forsberg et al. 2005). Over the last decade, high throughput devices (0.2–15 tons per hour) have been constructed and are in commercial use in Sweden, Norway and the Netherlands for the treatment of cereal and vegetable seeds (G. Forsberg, pers. communication).

Due to the comparatively long treatment durations required for pathogen inactivation (from a few days to 2 weeks or longer), seed treatments with dry heat often cause reductions in seed germination. However, they do not require sophisticated equipment and are therefore easy to apply. There are relatively few reports that claim successful control of seed-borne bacteria (e.g. Kubota et al. 2012) or fungi (e.g. Gilbert et al. 2005) by dry heat. In contrast, inactivation of viruses, both in vegetative propagation material and in seeds by dry heat treatments is well documented (Nyland and Goheen 1969; Grondeau and Samson 1994).

2.1.2 Other Physical Treatments

A seed treatment technology based on the application of low energy electrons (e-ventus®) has been developed in Germany. It is mainly effective against pathogens on the seed surface, like the spores of common bunt (*Tilletia caries*) or rye stripe smut (*Urocystis occulta*) (Jahn et al. 2005), and has also shown activity against a number of seed-borne vegetable pathogens. Various other physical effects such as high frequency fields, ultrasonic waves or microwaves have been studied for their suitability as seed treatments (Baker 1972; Bhaskara Reddy et al. 1998) but so far not been successful enough to be commercialized.

2.2 *Micro-organisms and Natural Products*

2.2.1 Micro-organisms

The basic mechanisms underlying biological control of plant pathogens are hyper-parasitism, suppression by antibiotics, lytic enzymes or other metabolites, and competitive exclusion. Micro-organisms may also elicit host defences; strains of root-colonizing bacteria have been identified as potential elicitors of plant host defences. In several instances, inoculation with plant-growth-promoting rhizobacteria (PGPR) resulted in control of multiple diseases caused by different pathogens (Pal and McSpadden Gardener 2006). The majority of micro-organisms used as biocontrol agents originate from plants, especially from the rhizoplane or from the rhizosphere. In recent studies it has been shown that induction of resistance and increased stress tolerance can also be triggered by seed-application of bacterial endophytes, i.e. strains originating from the interior of plants (Joe et al. 2012; Fürnkranz et al. 2012). For marketing as seeds treatment, the micro-organisms must be formulated in an appropriate way to ensure efficacy, storability and compatibility with existing agricultural technologies and practises. One way of delivery of micro-organisms to vegetable seed is by adding them during the priming process (‘biopriming’) (Jensen et al. 2007; Bennett et al. 2009; Pill et al. 2009). Microbial inocula or other natural products are not only added to seeds for technical reasons like plant growth promotion or disease control. They also provide the seed with a “green” label that is used in marketing.

2.2.2 Plant-Derived Products

Plants are a relatively untapped reservoir of different chemicals that can be used directly or serve as templates for the development of pesticides (Yoo et al. 2013). There is a large body of literature describing plants or plants constituents with antimicrobial properties. Activity against bacterial plant pathogens has been reported particularly for essentials oils (Iacobellis et al. 2005; van der Wolf et al. 2008; Mengulluoglu and Soylu 2012). Fungicidal activity of plant extracts has been shown against a large number of seed-borne fungi including members of important genera such as, e.g., *Fusarium*, *Alternaria* or *Colletotrichum* (Dal Bello and Sisterna 2010; Marinelli et al. 2012). The plants or plant parts may be used as powders or as extracts obtained by water or solvent extraction. However, the use of plant extracts in plant protection is often limited or infeasible due to phytotoxic properties of the preparations. Due to the general high sensitivity of germinating seed to external stimuli this holds especially true for the use as seed treatments. A typical example are the plant essential oils whose strong antimicrobial activity is often associated with adverse effects on the seed germination process (Dudai et al. 1999; Tworkoski 2002).

3 Examples of Non-chemical Seed Treatments for Control of Bacterial Pathogens

Due to a lack of chemical options, there has been more focus on non-chemical treatments for the control of seed-borne bacteria over recent years than for fungi. As bacterial pathogens are more important on vegetable crops than cereals there has inevitably been more work on these crops. Generally also, because of the great potential for secondary spread under favourable conditions, especially in transplanted vegetables (Roberts et al. 1999; Roberts et al. 2007), the seed health standards that need to be achieved are higher than for many fungal diseases.

3.1 Black Rot of Brassicas

Black rot of brassicas, caused by *Xanthomonas campestris* pv. *campestris*, is probably one of the most important diseases of brassicas worldwide. Hot water treatment has long been used as a seed treatment (Clayton 1924), but can result in reduced germination (Huber and Gould 1949). It can be very effective at reducing inoculum levels when optimised on a per seed lot basis. In recent experiments (Roberts et al. 2006), hot water and aerated steam consistently reduced seed infestation levels and seed-to-seedling transmission. Reductions in seed infestation of over 90 % and in transmission of over 63 % were achieved. Electron treatment was also examined, but this was less consistent and effective than hot water or aerated steam. Reductions in transmission have also been demonstrated for several microbial treatments; these have included experimental micro-organisms and commercial Bacillus products, Serenade and Subtilex (Roberts 2009) (Fig. 8.1). Thyme oil has also been shown to have potential as seed treatment for *X. campestris* pv. *campestris* on brassicas (van der Wolf et al. 2008; Roberts 2009) (Fig. 8.1).

3.2 Pea Bacterial Blight

Pea bacterial blight is caused by *Pseudomonas syringae* pv. *pisi*, and can cause significant losses particularly in over-wintered crops. The disease is primarily seed-borne and the use of disease-free seed is the main means of control (Roberts et al. 1996). Grondeau et al. (1992) examined a range of heat treatments in the form of hot water, hot humid air and dry heat treatments. Moist heat (50 °C, 100 % humidity, 48 h) reduced germination to un-acceptable levels and was not pursued further. Hot water (15 min, 55 °C) gave at least 75–84 % reductions in the percentage of seeds infested, and dry heat (65 °C, 72 h) gave 66–83 % reductions, without major effects on germination.

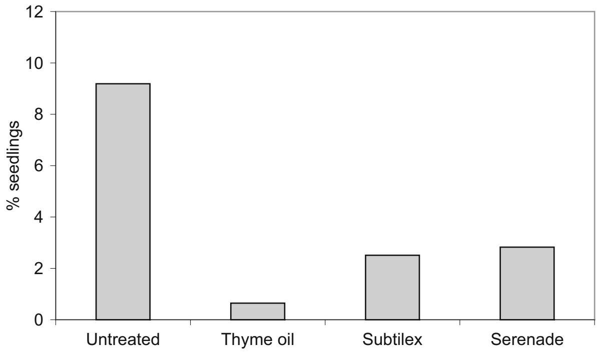

Fig. 8.1 Mean transmission of *Xanthomonas campestris* pv. *campestris* in three brassica seedlots grown as module transplants in the glasshouse, following treatment of the seed with biologicals/natural products. Values are the mean % of seedlings infested (i.e. contaminated or infected) (From Roberts 2009)

3.3 Bacterial Blotch of Cucurbits

Bacterial blotch of cucurbits is caused by *Acidovorax citrulli*, and can result in total crop loss in water melon crops (Latin and Hopkins 1995).

Rane and Latin (1992) obtained 80 % (naturally infested seed) and 96 % (laboratory infested seed) reductions in seed transmission with hot water treatment (50 °C, 20 min). Kubota et al. (2012) using dry heat (85 °C, 3–5 days) claimed complete disinfection. However, the maximum number of seeds tested was 300, implying tolerance standard of 1 % (see Roberts et al. 1999). Thus, with an initial infestation level of 25 %, they actually achieved a 96 % or greater reduction for melon and 97 % reduction for cucumber. As with dry heat for control of Tobamoviruses in tomatoes (see below), pre-drying seed to low moisture contents <5 % seems to reduce the likelihood of damage during heat treatment.

The use of a non-pathogenic (genetically modified) *A. citrulli* strain has been examined as a potential seed treatment (Johnson et al. 2011). They achieved an 82 % reduction in disease in growth chamber tests, but only a 38 % reduction in glasshouse tests. Seed treatment with a cell-free culture filtrate of a yeast has also resulted in a significant reduction in disease (Wang et al. 2009).

3.4 Black Chaff of Cereals

Black chaff and bacterial leaf stripe of cereals (barley, rye, wheat and triticale) are caused by the seed-borne bacterium *Xanthomonas translucens* pv. *translucens*. It has been reported from all continents, but is listed as a quarantine pathogen in some regions/countries. Outbreaks of the disease are sporadic and favoured by warm and moist conditions. Treatment of barley seed with dry heat (71–84 °C, 11 days) reduced bacterial numbers to undetectable levels from an initial level of over

10^6 CFU/g (Fourest et al. 1990). For a seed lot with a lower initial level, 4 days at 72 °C was effective. The authors recommended routine treatment at 72 °C for 5–7 days as the higher infestation level is unusual. However, Duveiller et al. (1997) comment that the method is not completely effective.

4 Examples of Non-chemical Seed Treatments for Control of Fungal Pathogens

4.1 Alternaria Diseases of Carrot

Leaf blight and black root rot of carrots are caused by *Alternaria dauci* and *A. radicina*. Both pathogens are seed-borne and also contribute to poor emergence. In an extensive study of non-chemical treatments for these pathogens, the efficacies of physical, microbial and natural products were compared (Koch et al. 2010). Treatments were evaluated in both controlled conditions and in field trials over several years, mainly on the basis of plant stand. A number of putative resistance inducers failed to give any control. In five field trials performed in four different countries significant improvements were obtained with hot water, aerated steam and electron treatment, two microbial treatments and an emulsion of thyme oil in water. The most effective treatments (hot water plus *C. rosea* IK726 and aerated steam treatment) resulted in almost a 100 % increase in plant stand (Fig. 8.2).

4.1.1 Black Leg of Brassicas

Phoma lingam (*Leptosphaeria maculans*) causes black leg and stem canker of brassicas. It can also cause death and damping-off of seedlings. Williams (1967) found that hot water treatment (50 °C, 25 min) reduced infestation levels by 89 %, but this was not adequate to prevent a serious outbreak of the disease for a seed lot with high levels of infestation (18 %). A number of non-chemical (microbial) treatments and hot water have also been investigated more recently (Clarkson and Roberts 2011). The best treatments: hot water (50 °C, 30 min), thyme oil, Serenade (*Bacillus subtilis*) and an experimental microbial product significantly reduced seed infestation levels compared to the untreated control and were as effective as thiram. Hot water treatment was the most effective and resulted in an 88 % reduction in transmission. However, it also resulted in a significant reduction in emergence and an increase in damping-off caused by *Pythium* spp.

4.2 Common Bunt of Wheat

Common bunt of wheat is caused by *Tilletia caries* and *T. laevis*. Individual grains are replaced by masses of black spores which are dispersed to healthy grains at harvest and during grain handling. For effective control, high seed health standards

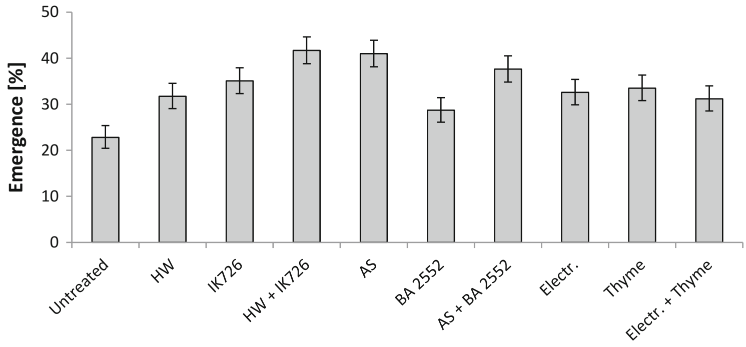

Fig. 8.2 Effect of selected seed treatments or treatment combinations on establishment of carrot plants developing from seeds naturally infected with *Alternaria dauci* and *A. radicina*. Means of five field experiments performed in 2006 in Sweden, UK, Italy and Germany. Error bars show approximate 95% confidence intervals; means with non-overlapping confidence intervals were considered to be significantly different (*HW* hot water; IK726: *Clonostachys rosea* IK726; *AS* aerated steam; BA2552: experimental formulation of *Pseudomonas chlororaphis* MA342; *Electr* electron treatment; Thyme: Emulsion of thyme oil in water) (Koch et al. 2010)

are considered necessary. Thresholds for spore load recommended in different countries vary between <1 and 20 spores per seed and should be adapted to the susceptibility of the variety (Waldow and Jahn 2007).

Seed treatment with different organic substances such as skimmed milk powder or wheat flour has been shown to be effective experimentally (Becker and Weltzien 1993). However, one of the obstacles for commercialization is that technologies such as seed pelleting are required to apply the needed large amounts of material to the seed. A product based on yellow mustard powder (Tillecur®) is commercialized in several European countries where it is used primarily for bunt control in organic farming (Waldow and Jahn 2007).

Numerous experimental and commercialized micro-organisms have been examined as seed treatments on wheat against common bunt (Hökeberg et al. 1997; Koch et al. 2004; Koch et al. 2006; Goates and Mercier 2011). However, so far only the bacterium *Pseudomonas chlororaphis* MA342 has been commercialised and is marketed in Europe as an oil-based formulation (Cedomon®) for hulled seeds (like barley) and as a water-based formulation (Cerall®) for non-hulled seeds (like wheat). In field trials with spelt (*Triticum spelta*), treatment with Cedomon® reduced disease incidence by almost 90 % and with Cerall® on dehulled spelt an 80 % reduction in disease incidence was recorded (Krebs 2010).

Control of common bunt with levels almost equivalent to chemical seed treatments haves been reported for both electron treatment (mean reductions of 87–94 %) (Jahn et al. 2005) and aerated steam (95 % reduction) (Forsberg et al. 2005).

4.3 Fusarium *spp. and* Microdochium *spp. on Small-Grain Cereals*

Fungal pathogens belonging to species of *Fusarium*, *Microdochium*, *Phaeosphaeria*, *Pyrenophora* and *Rhynchosporium* affect primarily germination and seedling health of small grain cereals. They are mostly located in the pericarp (outer layer of the grain). In the following, options for non-chemical control of this group of seed-borne pathogens will be explained using the example of *Fusarium* spp. and *Microdochium* spp. Both cause reductions in germination and seedling losses. Under a snow cover, *Microdochium majus* and *M. nivale* may cause snow mould. *Fusarium* spp. and *Microdochium* spp. may under favourable conditions also penetrate deeper into the endosperm or even colonise the embryo.

Seed infections with *Fusarium* spp. and *Microdochium* spp. have been successfully controlled with warm water (45 °C, 2 h; increase in plants/m row by 160 %; Vogelgsang 2013). Positive effects were also obtained with aerated steam treatments (increase in crop density of 20 %, chemical: 28 %; Forsberg et al. 2005). Treatment with dry heat at 70 °C for 5 days was recommended for eradicating *F. graminearum* from wheat seeds (Clear et al. 2002; Gilbert et al. 2005). Microwave irradiation has also been shown to reduce the percentage of wheat seed infected with *Fusarium graminearum* by at least 74 % (Bhaskara Reddy et al. 1998).

Seed treatment with fungal antagonists belonging to species of *Trichoderma*, *Gliocladium* and *Penicillium* were reported to reduce foot and root rot caused by *F. culmorum* in field experiments in Italy, although to a lesser extent than the chemical (Roberti et al. 2000). In seed tray tests with wheat seed lots naturally infected with *Fusarium* spp. significant increases in the number of healthy seedlings were obtained with *Streptomyces antimycoticus* strain FZB53 (Koch et al. 2006). Analytical studies indicated that the activity was largely due to an unidentified polyether antibiotic and geldanamycin produced by the antagonist (Koch et al. 2008). In field and greenhouse experiments using seeds artificially inoculated with *F. culmorum* disease indices on seedlings of barley and wheat were repeatedly reduced by more than 80 % by seed treatment with *Clonostachys rosea* IK726 (syn. *Gliocladium roseum*) (Jensen et al. 2000). Chitosan, a polymer of β-1,4 linked D-glucosamine applied to wheat seed infected with *F. graminearum* significantly improved seed germination and at the higher concentrations tested inhibited fungal transmission to the primary roots of germinating seedlings by >50 %. The observed effects were attributed to activation of plant defence, although a partial contribution of the antifungal properties of chitosan could not be totally ruled out (Bhaskara Reddy et al. 1999).

Preparations from Chinese galls (obtained from *Rhus chinensis*) have recently been shown to have potential to control seed-borne *Microdochium majus* (Vogelgsang et al. 2013). In vitro, Chinese galls at a concentration of 1 % inhibited *M. majus* conidial germination almost as effectively as the synthetic fungicide Pronto® Plus (spiroxamine + tebuconazole). In field experiments, over three

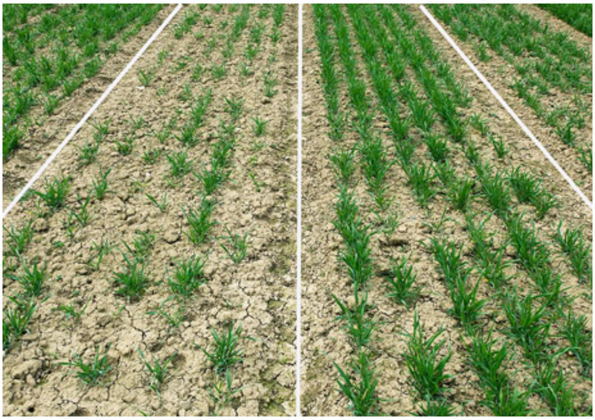

Fig. 8.3 Emergence of wheat from kernels naturally infected with *Microdochium majus*. *Left*: Untreated control, *Right*: seed treated with Chinese galls + adhesive (Courtesy of H. Krebs, Agroscope, Zürich)

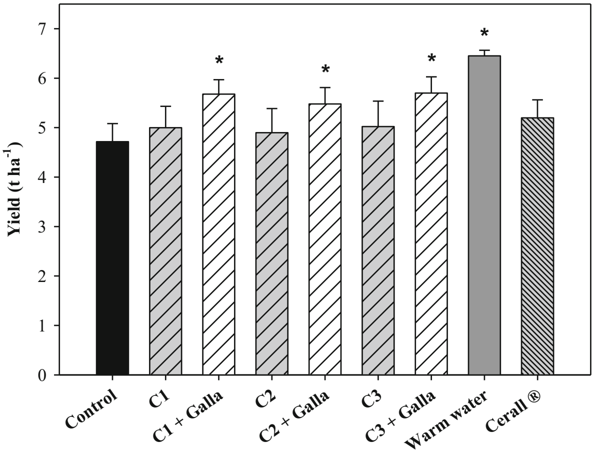

Fig. 8.4 Effect of seed treatment of wheat infected with *Microdochium majus* on grain yield in field experiments 2009–2011. C1, C2, C3: different application procedures of the adhesive; Galla: *Rhus chinensis* (2 g per 100 g seeds); warm water: 45 °C, 2 h; Cerall: 1 ml per 100 g seeds. Means and standard errors of means. *Asterisks* indicate significant differences (α=0.05) to the control. (Vogelgsang et al. 2013, with permission)

years, treatment of infected wheat kernels with three different formulations of Chinese galls resulted in significant increases in emergence (Fig. 8.3) and yield (Fig. 8.4). Chinese galls are known to contain tannin-derived components with low pH, but whether these have a role in the antifungal activity is not conclusive (Vogelgsang et al. 2013).

4.4 Loose Smuts of Barley and Wheat

Due to their localization in the seed embryo and early colonization of the apical meristem (Wunderle et al. 2012), the loose smut fungi *U. nuda* and *U. tritici* are particularly difficult to control. To the authors' knowledge, effective sanitization of infected seed lots by non-chemical methods is only possible by thermal treatment in water. The effect obtained with aerated steam was only partial and clearly lower than with the standard chemical seed treatment (Forsberg et al. 2005). A range of plant extracts and microbial antagonists with in-vitro activity against germinating spores of *U. nuda* have been screened in field trials, but none gave satisfactory control (Koch, unpublished results).

4.5 Diseases of Sorghum

In Nigeria, Ghana and Burkina Faso *Fusarium*, *Curvularia* and *Phoma* are common on sorghum (*Sorghum bicolor*) seed; they affect seed germination and seedling health (Zida et al. 2012). *Phoma sorghina* (teleomorph *Leptosphaeria sacchari*) is primarily located in the seed coat but can also be found in the endosperm and embryo, although at lower frequency (Schémaeza et al. 2012). Among different aqueous plant extracts tested for activity against *P. sorghina*, the most effective were those from *Cymbopogon citratus* (30 % W/V, treatment duration 24 h) and *Eclipta alba* (10 % W/V, treatment duration 20 h). An aqueous extract of *Yucca schidigera* showed antifungal activity against *P. sorghina*, *Fusarium* spp., *Cochliobolus lunatus* and *Cladosporium* spp. and increased seedling emergence and seedling vigour. The activity was suspected to be due to saponins present in the extract (Wulff et al. 2012).

Similarly, in field experiments using inoculated seeds, treatment with dried powder from the berries of African soapberry (*Phytolacca dodecandra*), known to contain saponins, reduced the disease incidence of covered kernel smut (*Sporisorium sorghi*) (Fig. 8.5) and loose kernel smut (*S. cruentum*) by 82–92 % (Tegegne and Pretorius 2007). A crude extract from aerial parts of *Agapanthus africanus* controlled both smuts completely (Tegegne et al. 2008). Results of seed treatment experiments performed in the glasshouse indicated a high activity against *S. sorghi* also for Tillecur®, an aqueous extract of *Quillaja saponaria* and *Trichoderma harzianum* (Moharam 2010).

4.6 Diseases of Rice

A large number of plant extracts have been screened for fungicidal activity against rice pathogens (e.g. Mohana et al. 2011), but few studies involved testing on infected seed. Garlic extracts applied to rice seeds were as effective at reducing the incidence of different seed-borne pathogens as the synthetic chemical reference fungicide (Yeasmin et al. 2012). Broad spectrum fungicidal activity was also recorded for the essential oils of *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* applied as emulsions in 0.1 % agar to rice seeds. The treatments

Fig. 8.5 Sorghum panicle with healthy seeds and sori of covered kernel smut. The sori are crushed at harvest and adhere to the seed surface from where they infect the germinating plant

reduced seed infection with *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme* in blotter tests and seed to seedling transmission in pot experiments by 76–95 % (Nguefack et al. 2008).

In Japan, *Trichoderma asperellum* SKT-1 (Ecohope®) and *Talaromyces flavus* SAY-Y-9401 (Tough-block®) are registered as seed treatments for control of seed-borne *G. fujikuroi* (Nagayama et al. 2007; Kato et al. 2012) and other seed-borne pathogens of rice.

5 Examples for Non-chemical Seed Treatments for Control of Viruses

There are relatively few examples of non-chemical seed treatments for viruses. Probably this is because, for many viruses, the virus must be present in the embryonic axis for transmission to occur, and presents a difficult target for treatment without damaging the seed.

5.1 Solanacae and Tobamovirus

The tobamoviruses TMV (tobacco mosaic virus) and ToMV (tomato mosaic virus) are mechanically transmissible and able to retain their infectivity in the seed coat of dry tomato seeds. Dry heat (80 °C, 24 h) reduced the transmission of TMV in tomato to undetectable levels in most, but not all, seed lots (Laterrot and Pécaut 1968). In the one seed lot where transmission was detected, a reduction of 70 % was achieved. Dry heat treatment (78 °C, 2 days) reduced the levels of ToMV in tomato seed by over 95 % (based on the number of local lesions in a host test) without detrimental effects on germination after storage for 12 months (Green et al. 1987). Prior to heat treatment seeds were brought to a moisture content of between 6 % and 8 %. Dry heat (80 °C, 24 h) has also been shown to reduce levels of ToMV in pepino seeds to undetectable levels (<3 %) but led to a reduction in germination that varied between species (Prohens et al. 1999).

5.2 *Melon Necrotic Spot Virus*

Melon necrotic spot virus is an important pathogen of glasshouse and field-grown melons and cucumbers. The effect of dry heat at 70 °C for 3–6 days on germination and virus transmission was examined by Herrera-Vásquez et al. (2009). The best treatment (6 days at 70 °C) gave at least 80–86 % reductions in transmission (although the authors interpreted this as total eradication) with little effect on germination.

6 Summary

There are a number of non-chemical seed treatment options for the control of seed-borne diseases. No treatment can be guaranteed to completely eliminate the target pathogen, and claims of 'eradication' or 'complete control' should be regarded with some suspicion. Physical treatments with hot water, aerated steam, or dry heat have successfully been applied to a range of crops against a range of target pathogens and are in commercial use. The level of success achieved depends on the location of inoculum and optimisation of the treatment parameters for different species and seed lots. Hot water and aerated steam seem to perform relatively better on small seeds when the inoculum is mostly superficial, whereas dry heat may be better for viruses, with larger seeds and/or where the inoculum is more deep-seated. Where feasible, the efficacy of treatment should be checked with a post-treatment seed test. An advantage of the physical treatments is that they can target a number of pathogens at the same time, but the non-specific nature and potential for sub-lethal damage of seed can potentially give rise to other problems such as increased susceptibility to soil-borne pathogens. Expansion of commercial use of the physical treatments has been hampered by a number of factors such as: problems associated with the batch treatment of large bulks of seeds, the difficulty of applying precise treatments, the need for re-drying after hot water treatment, a lack of commercial equipment, and the need for optimisation of treatment parameters for each seed lot. Recently-developed innovative technologies (Thermoseed®, e-ventus®) are overcoming some, but not all, of these obstacles.

Microbial seed treatments have had much more variable levels of success. It is interesting to note that many of the microbial antagonists that protect against root or foliar pathogens were reported to have been applied by seed treatment. The literature specifically describing control of seed-borne diseases by microbial antagonists is nevertheless limited compared to the huge number of reports on microbial control of other kinds of plant diseases. Only a few microbial seed treatments for control of seed-borne pathogens are commercially available. This may be due to a lack of research on the one hand but more often due to commercial constraints like development costs in relation to market size, the feasibility of mass-production, formulation, and general difficulties associated with the registration of microbials as plant protection products. Also, where microbial treatments have been originally developed for foliar application, there seems little incentive to seek approval as a seed treatment, as the potential market volume is much lower. However, with changes to legislation in the EU and following several recent take-overs of smaller biocontrol-focused companies by large multi-nationals, it is possible that the rate of progress will increase over the next few years.

Despite many reports of bactericidal and fungicidal effects of compounds from plants in the literature, the use of botanicals as seed treatments is still rare. As with microbial products, economic considerations like cost of registration and limited attractiveness for the market are likely to be the main reasons. Also, companies find it difficult to claim intellectual rights for products from plants with published antimicrobial properties. An alternative to commercialization of botanical seed treatments by companies could be self-preparation by the user, provided this would be in line with legislation (for example use of plant material as basic substance according to regulation EC No. 1107/2009). The use of non-chemical seed treatments based on natural products such as powders or extracts from local plants could also be a sustainable solution particularly for many developing countries where chemical seed treatments are unaffordable or not available to the farmer.

Finally, we conclude that for many seed-borne pathogens, there are potentially effective non-chemical seed treatment alternatives to synthetic chemicals available or that could be developed. The fact that they haven't been exploited more fully seems to be largely due to a lack of commercial incentives; this may change with the increasing concerns about the safety of synthetic chemical pesticides.

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