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Species of *Trichoderma* and *Aspergillus* as Biological Control Agents against Plant Diseases in Africa

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Introduction

Plant diseases cause significant losses in yield and quality of crops in Africa, where a growing population needs more food and income from agriculture-related activities. Sound plant health management is key to sustainable management of diseases. Integrated plant health management has several interactive facets such as appropriate crop germplasm, sound cultural practices and crop management, biologically based inputs and synthetic chemicals. The complex and interactive nature of good crop management requires a robust understanding of the agroecosystem in which individual components of plant health are nested. For example, crop rotation or tillage practices may be less amenable to manipulation in a perennial cropping system than in an annual cropping system where the field site can be changed periodically. Host resistance reinforced by biological control can be a useful component of management in perennial systems (McSpadden *et al.*, 2002). Economics of crop production, economics of losses caused by a disease in a specific situation, and ease and cost of applying disease management methods determine the level of intervention that a farmer is willing to make to realize gains from farming. Usually, the primary foundation for disease control is manipulation of the physical environment and utilizing host resistance. Biological control and synthetic fungicides provide further support to disease management. However, use of fungicides is being discouraged due to economic reasons and growing concern for environment and safety issues. Biological control is potentially a sustainable solution to plant diseases in African agriculture since its effect is long-term with few, if any, undesirable side-effects.

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Biological control agents act against plant pathogens through different modes of action. Antagonistic interactions that can lead to biological control include antibiosis, competition and hyperparasitism (Cook and Baker, 1983). Competition occurs when two or more microorganisms require the same resources in excess of their supply. These resources can include space, nutrients and oxygen. In a biological control system, the more efficient competitor, i.e. the biological control agent, out-competes the less efficient one, i.e. the pathogen. Antibiosis occurs when antibiotics or toxic metabolites produced by one microorganism have direct inhibitory effect on another. Hyperparasitism or predation results from biotrophic or necrotrophic interactions that lead to parasitism of the plant pathogen by the biological control agent. Some microorganisms, particularly those in soil, can reduce damage from diseases by promoting plant growth or by inducing host resistance against a myriad of pathogens (Hutchinson, 1998; Cook, 2000; Kerry, 2000). Efficient biological control agents often express more than one mode of action for suppressing the plant pathogens.

Several naturally occurring microorganisms have been identified as biological control agents of plant pathogens. This chapter deals with biological control of fungal diseases of crops with fungal species belonging to the genera *Trichoderma*, *Fusarium* and *Aspergillus* purely in the African context. Discussion on the use of *Fusarium* species for the control of the obnoxious witchweed (*Striga hermonthica* (Del.) Benth.) in maize and sorghum, and burrowing nematode (*Radopholus similis* (Cobb) Thorne) in banana can be found elsewhere in this volume (see Berner *et al.* for *Striga* and Sikora *et al.* for *R. similis*).

Disease Control Using *Trichoderma* Species

The antagonistic ability of *Trichoderma* species was discovered 70 years ago (Weindling, 1932). *Trichoderma* spp. are now the most common fungal biological control agents that have been extensively researched and deployed throughout the world. The primary mechanism of antagonism in *Trichoderma* is mycoparasitism. Lytic activity is the key feature responsible for the expression of mycoparasitism against several fungal pathogens (Chet, 1987). *Trichoderma* spp. are also good competitors in soil, and producers of volatile and non-volatile antibiotics to suppress target pathogens (Chet, 1987). Because of their effectiveness and ease of production for commercial application, at least nine commercial biological control products based on *Trichoderma* species are manufactured and marketed in Belgium, Sweden, Israel, USA, Denmark, India and New Zealand for use on several crops (Navi and Bandyopadhyay, 2002). In Africa too, considerable research has been done on biological control potential of *Trichoderma* spp. against several fungal pathogens that attack seeds, seedlings, roots, stems and leaves of several crops. Some of the diseases that can be potentially controlled by *Trichoderma* species are listed in Table 13.1. Two specific examples are highlighted below to illustrate the potential biological control of seed and seedling blight of cowpea and stalk and ear rot of maize.

Table 13.1. Evidence for successful experimental use of *Trichoderma* spp. as a biological control agent of various crop diseases in Africa.

Host	Disease	Pathogen	Species of *Trichoderma*	Reference
Cowpea	Damping-off	*Macrophomina phaseolina*	*T. harzianum*, *T. koningii*	Adekunle *et al.* (2001)
Cowpea	Web blight	*M. phaseolina*	*T. koningii*	Latunde-Dada (1991)
Cowpea	Leaf smut	*Protomycopsis phaseoli*	*T.* spp.	Adejumo *et al.* (1999)
Maize	Storage seed rot	*Gibberella fujikuroi* and *Aspergillus flavus*	*T.* spp.	Calistru *et al.* (1997)
Soybean	Brown stem rot	*Phialophora gregata*	*T. harzianum*	Yehia *et al.* (1994)
Potato	Stem canker and black scurf	*Rhizoctonia solani*	*T. harzianum*, *T. koningii*	Abada and Abdel-Aziz (2002)
Potato	Leak	*Pythium aphanidermatum*	*T. harzianum*	Triki and Priou (1997)
Tomato	Southern blight	*Sclerotium rolfsii*	*T. koningii*	Latunde-Dada (1993)
Tomato	Basal stem rot	*S. rolfsii*	*T. viridae*	Wokocha (1990)
Lucerne	Damping-off, wilt and root rot	*R. solani* and *Fusarium oxysporum*	*T. harzianum*	Hassanein *et al.* (2000)
Strawberry	Grey mould rot of fruits	*Botrytis cinerea*	*T. harzianum*	El-Zayat *et al.* (1993)
Cucumber	Damping-off	*R. solani*	*T.* spp.	Askew and Laing (1994)
Sugar beet	Damping-off and root rot	Several fungi	*T. harzianum*	Abada (1994)
Table beet	Damping-off	*P. aphanidermatum*	*T. harzianum*	Abdalla and El-Gizawy (2000)
Avocado	Root rot	*Phytophthora cinnamomi*	*T. harzianum* *T. hamatum*	McLeod *et al.* (1995)
Garlic	White rot	*Sclerotium cepivorum*	*T. harzianum*	Rahman *et al.* (1998)
Tobacco	Damping-off and root rot	*R. solani* and *F. solani*	*T. harzianum*	Cole and Zvenyika (1988)

Seed and seedling blight of cowpea

Several diseases affect cowpea (*Vigna unguiculata* Walp., *Papilionaceae*) during its growth and development from the time the seed germinates in soil to the time when seeds are produced and harvested. Some of these diseases are amenable to biological control while others are not. Seed decay and seedling damping-off cause serious losses in cowpea (Emechebe and Shoyinka, 1985). Among the several pathogens associated with these seed and seedling diseases, *Macrophomina phaseolina* (Tassi.) Goid is prevalent in the Sudano-Sahelian areas where cowpea frequently suffers from moisture stress. In

addition to seedling diseases, the pathogen also causes ashy stem blight or charcoal rot. *M. phaseolina* is extremely plurivorous and causes diseases in more than 300 plant species. This soil-inhabiting fungus survives for several years as free sclerotia in soil and in infected plant debris. Under favourable infection conditions, the pathogen propagules around the spermosphere and hypocotyl colonize the seed, hypocotyl and epicotyl leading to pre- and post-emergence damping-off of seedlings. In other words, seed decay and damping-off appear early during plant growth in a localized part of the plant. Therefore, disease control methods targeting the seeds have been useful in managing the disease. Seed treatment with systemic fungicides such as benzimidazole compounds is effective in controlling the disease (Kataria and Sunder, 1985). However, these fungicides are not generally available to resource-poor farmers who are the major cowpea producers.

Biological control of seed decay and damping-off of cowpea has been demonstrated using species of *Trichoderma* as antagonists (Adekunle *et al.*, 2001). *T. harzianum* Rifai, *T. koningii* Oudem and an unknown species of *Trichoderma* were tested at different doses to determine the efficacy of the antagonists. The plant stand was significantly improved when seeds were treated with *T. harzianum* and *T. koningii* compared with untreated seeds. Although the protection with the antagonists was lost over time, *T. harzianum* was more effective than *T. koningii* since the protection with the former lasted longer than the latter. Several formulations of the antagonists were also evaluated. The antagonists were grown in liquid culture, harvested, dried in an oven at 30°C for 48 h, and powdered in a blender. The powdered antagonist was suspended in water, and the aqueous suspension used to prepare two formulations: suspension with a sticker (Tween 20) and with cassava starch as an adhesive. The powdered antagonist was also transformed into concentrated slurry with water and uncooked cassava starch powder. Seeds were treated for different duration with each of these formulations. Generally seed treatment with the slurry formulation was not effective in reducing the disease, while soaking the seeds for 10–40 min in the aqueous suspension of the antagonists amended with cassava starch significantly reduced the disease.

Seed application requires only a small quantity of a biological control agent and can be easily combined with fungicidal seed dressing to enhance the efficacy of both for controlling diseases (Harman and Taylor, 1990; Cook, 2000), as has been suggested for cowpea–*M. phaseolina* system (Alagarsamy and Sivaprakasam, 1988). Of course, the fungicide added in the biological control formulation should not be toxic to the biological control agent nor should it be expensive.

Seed dressing is a technology appropriate for African farming systems, and cottage industry production units have been shown to be economically feasible for meeting local or small-scale demands (Cherry *et al.*, 1999). The feasibility of a local biopesticide with *Trichoderma* depends on several factors. The raw materials, adhesive and production substrates need to be plentiful and cheap. The *Trichoderma* isolate would have to be quite robust and grow quickly on local substrate such as rice hulls or coconut shells. The risk of inadvertently increasing potential human pathogen along with the biological control agent

must be very low. The dose response cannot be too stringent or safeguards would have to be developed for 'under-dosing' or 'over-dosing'. If these conditions can be met, then development of *Trichoderma*-based seed treatment in Africa will be attractive. *Trichoderma* populations in soil would probably increase with external introduction, particularly in acidic soils in West Africa.

Stalk and ear rot of maize

Species of *Fusarium* belonging to the section *Liseola* can cause seedling diseases, root rots, stalk rots and ear rots of maize in the field, as well as postharvest storage rots. *Fusarium verticillioides* (Saccardo) Nirenberg (*F. moniliforme* J. Sheldon) and other anamorphs belonging to the teleomorph *Giberella fujikuroi* (Sawada) Ito in Ito and K. Kimura, are most frequently isolated from maize plants. Interest in the disease stems from the concern that infection of grain by *G. fujikuroi* can lead to loss of grain quality and potential production of fumonisin and other harmful mycotoxins (Munkvold and Desjardins, 1997). *G. fujikuroi* comprises several mating populations. Among these, those belonging to mating population A are considered as *F. verticillioides*. Other species infecting maize are *Fusarium proliferatum* (Mats.) Nirenberg ex Gerlach and Nirenberg and *Fusarium subglutinans* (Wollenw. and Reinking) Nelson, Toussoun and Marasas.

Members belonging to mating population A are more potent producers of fumonisin and are found more frequently on maize compared with mating population F (e.g. *Fusarium thapsinum* Klittich, Leslie, Nelson and Marasas), which produces little or no fumonisin (Leslie *et al.*, 2001). *F. verticillioides* is closely associated with maize throughout the plant's life living as an endophyte within the plant right from seedling to grain harvest, often without causing any visible symptoms. While many infected plants remain free of symptoms, damage in others can be dramatic. The fungus is transmitted through seed infection that results from vertical spread of the endophytic phase from stalk to the grain. Seed infection cannot be controlled by fungicide sprays since it is transmitted internally through the plant. The fungus also survives in plant debris on the soil surface, and free ambient spores can infect the stalk through the adventitious roots and the ear via the silk channel. Insects play an important role in moving the fungus and opening infection sites in maize stalks and ears (Munkvold and Desjardins, 1997). At the same time, *F. verticillioides* has been shown to attract insects to the plant (Schulthess *et al.*, 2002) resulting in a critical feedback loop of infection and damage. Thus, control of endophytic *F. verticillioides* may exert a collateral effect of reducing attractiveness and susceptibility of the maize plant to insects.

Fumonisin-related restrictions for trade have led to a renewed interest in finding strategies to reduce the levels of contamination of maize with the toxin. Currently, host-plant resistance, insect-pest control and good storage practices are the major strategies for stalk rot and ear rot management. Biological control of stalk rot (Sobowale, 2002) and storage rot (Bacon *et al.*, 2001) by means of *Trichoderma* spp. has also been explored in order to reinforce other manage-

ment tactics. Sobowale (2002) isolated 52 fungi from different parts of maize plants and tested these against *F. verticillioides* initially in *in vitro* tests. Seven of these fungal isolates, all belonging to *Trichoderma* spp., were further tested against *F. verticillioides* in artificially infested stalks. *T. harzianum* and *Trichoderma pseudokoningii* Rifai were found to occupy the same niche as *F. verticillioides* and were able to competitively displace the pathogen. These two antagonists were able to move within the stalk to internodes further away from the point of introduction to the sites where *F. verticillioides* existed. Significantly, it appeared as if the antagonists sensed and tracked *F. verticillioides* in the stalks. Recovery of the pathogen from stalks co-inoculated with antagonists was significantly lower than from stalks in which the antagonists were absent. However, introduction of the antagonists into stalks was ineffective and did not protect against accumulation of fumonisins in grains.

The potential of *T. harzianum* and *Trichoderma viride* Persoon: Fries to reduce mycotoxin-producing potential of *F. verticillioides* in grain store has been further explored by Bacon *et al.* (2001) and Calistru *et al.* (1997). The latter authors suggested that the aggressive behaviour (towards *G. fujikuroi* demonstrated by *Trichoderma* spp. could be partly explained by the liberation of extracellular enzymes by these fungi. An isolate of *T. viride* showed amylolytic, pectinolytic, proteolytic and cellulolytic activity. Although management of *Fusarium* stalk rot and grain spoilage in storage are potentially amenable to biological control, more work is required to test the biological activity of various agents, the different potential delivery mechanisms for biological control agents, and the practical feasibility and economy of this approach.

Disease Control Using *Aspergillus* spp.

Species of *Aspergillus* are almost ubiquitously present in soils of tropical areas. Most species of *Aspergillus* are not of much consequence in agriculture, but some species of *Aspergillus* are found in plant products, particularly oil-rich seeds. Contamination of seeds with highly poisonous aflatoxins results from the presence of toxigenic strains of four species of *Aspergillus*: *A. flavus* Link:Fr. (Plate 40), *A. parasiticus* Speare, *A. nomius* Kurtzman, Horn and Hesseltine and *A. bombycis* Peterson, Ito, Horn and Goto (Peterson *et al.*, 2001), each producing a combination of different types of aflatoxins. In Nigeria, Bénin and Togo, for instance, maize is consumed and stored across all agroecological zones. Depending on agroecology, crop management and length of storage, aflatoxin contamination levels averaging >100 p.p.b. have been recorded in up to 50% of grain stores sampled (Hell *et al.*, 2000a; Udoh *et al.*, 2000) and in some samples collected in Bénin, extremely high amounts of up to 2500 p.p.b. of aflatoxin were detected (Sétamou *et al.*, 1997). These levels of aflatoxin are much higher than the maximum permissible limit of 20 p.p.b. in food and feed.

Aflatoxins in Africa have impact on human and animal health and on trade. Thus, alfatoxin has been reported to be associated with exacerbation of the energy malnutrition syndrome Kwashiorkor in children and vitamin A malnutrition in animals, and many other problems (Hall and Wild, 1994). In various ani-

mal models, in addition to being hepatotoxic, aflatoxin causes significant growth faltering and is strongly immune-suppressive at weaning. It has been recently shown that 99% of all children weaned from mother's milk to maize-based diets in Bénin and Togo had aflatoxin in their blood, indicating ingestion of aflatoxin-contaminated food (Gong *et al.*, 2002). Aflatoxin exposure in children was associated with stunting (a sign of chronic malnutrition) and being underweight (an indicator of acute malnutrition). In addition to the direct public health impact, there is ample evidence of negative impact on trade in Africa owing to aflatoxin, particularly with respect to trade with the European Community. Therefore, a reduction of aflatoxins in maize and groundnut through appropriate integrated management in field and store is extremely important to reduce losses, increase rural incomes, and improve health and wellbeing of people.

A. flavus invades and infects developing seed of maize and groundnut in the field before harvest, and mature seeds during harvest, and in storage. Preharvest contamination with aflatoxins is aggravated by drought stress and elevated temperature during seed maturation. Damage by insects is another important predisposing factor by providing injury sites through which *A. flavus* can invade seeds in the stores and in the field (Sétamou *et al.*, 1998; Hell *et al.*, 2000a,b). *A. flavus* populations are genetically highly diverse and are composed of large numbers of vegetative compatibility groups (VCGs), sometimes within a restricted geographic area. VCG composition of native strains within a field may, however, be of only minor importance in predicting the efficacy of a non-aflatoxigenic biological control strain (Horn *et al.*, 2000). Populations of the *Aspergillus* group *flavi* also contain mixtures of members that can produce copious amounts of aflatoxins (aflatoxigenic) and those that cannot produce at all or produce insignificant amounts of aflatoxins (non-aflatoxigenic) (Egel *et al.*, 1994; Cotty, 1997). Diversity in the population of *Aspergillus* spp. also exists with respect to their size of sclerotia. S-strain (those producing small sclerotia) isolates in the USA produce high amounts of aflatoxin B, while S-strains from Africa produce both aflatoxin B and G (Cotty and Cardwell, 1999). L-strains (with large sclerotia) on both continents produce a range of aflatoxin B from none to very high. Subsequent work suggested that small sclerotial S-phenotype is not predictive of aflatoxin production (Geiser *et al.*, 2000). Toxigenicity of *A. flavus* is apparently unrelated to a strain's ability to colonize and/or infect living or dead plant tissues (Cotty and Bayman, 1993).

In the USA, biological control has been used to reduce aflatoxin contamination in various crops such as cotton (Cotty, 1994), groundnut (Dorner *et al.*, 1998) and maize (Brown *et al.*, 1991; Dorner *et al.*, 1999). This technique involves the application to soil of a non-aflatoxigenic biological control strain of *A. flavus* or *A. parasiticus*, resulting in a high population density that allows the biological control strain to compete effectively with the native aflatoxigenic strains during invasion under conditions favourable for aflatoxin contamination. Invasion of a seed in soil (e.g. groundnut) solely by the biological control agent would be expected because of its high density relative to the wild-type strain in the soil. This would result in less aflatoxin contamination. For maize and cottonseed, the high population of the non-aflatoxigenic biological control

strain in soil produces abundant spores on the soil surface that become airborne to infect grains and seeds (Horn *et al.*, 2001).

The potential to reduce aflatoxin contamination in maize using the biological control tactics mentioned above has been evaluated in Bénin, where 90% of the *Aspergilli* are *A. flavus* (Cardwell and Cotty, 2002). The non-aflatoxigenic strains of *A. flavus* (BN22 and BN 30 from Bénin, and AF36 from the US) were tested against aflatoxigenic strains of *A. flavus* (BN40 from Bénin and AF13 from the US) and *A. parasiticus* (BN48) *in vitro* (Cardwell and Cotty, 2000). For these *in vitro* trials, maize kernels were dipped in 1×10^6 conidia ml^{-1} suspension of one *Aspergillus* spp. or strain and allowed to dry before repeating the dip with either the same or another strain. Kernels were incubated in a saturated environment at 30°C for 5 days, dried at 40°C for 5 days, crushed, extracted in acetone and aflatoxins quantified using thin-layer chromatography (TLC) and a scanning densitometer. All non-aflatoxigenic isolates significantly reduced toxin production by the African *A. parasiticus* isolate BN48. The American non-aflatoxigenic isolate AF36 was effective against the American aflatoxigenic isolate AF13, but not the aflatoxigenic African S-strain, BN40 suggesting that there may be specificity of action of some non-aflatoxigenic strains. The African non-aflatoxigenic L-strain BN30 was the only isolate that reduced toxin production by the aflatoxigenic African S-strain, BN40.

Field tests were also conducted in Bénin in 1998 and 1999 using non-aflatoxigenic African L-strain BN30 and aflatoxigenic African S-strain, BN40 (Cardwell and Cotty, 2000). Silks of 60-day-old maize plants were dipped in 1×10^6 conidia ml^{-1} suspension of the aflatoxigenic S-strain BN40 or water (control) and covered overnight with a paper bag. Seven days later, the silk was dipped again either in the suspension of the same strain or non-aflatoxigenic L-strain BN30. Ears were harvested 2 weeks after maturity and shelled. Grain was milled, extracted with chloroform and aflatoxins quantified using TLC. In these field trials, toxin production was significantly reduced and was not different from the water control (Fig. 13.1A and B). The second isolate inoculant consistently influenced toxin production in the field. In fact it had more impact on toxin production than the first, which was judged to be counter-intuitive. If the mechanism of toxin reduction was competitive displacement, as is the hypothesis in the cotton model, it was expected that the first inoculant would occupy the kernel and the second would be excluded. Nevertheless, this study confirms that non-aflatoxigenic strains are effective in reducing aflatoxin production in maize by competitively displacing the aflatoxigenic strains of *A. flavus* and *A. parasiticus*.

Biological control of *A. flavus* in Bénin is still at a rudimentary stage. Across the country, many isolates of *A. flavus* strains have been identified (Cardwell and Cotty, 2000). There seems to be a gradient in the distribution of these strains from north to south in Bénin (Cardwell and Cotty, 2002), with the north having more of the highly toxigenic S-strains and the south more of the less toxic L-strains (Cardwell and Cotty, 2002). Further work has to determine whether the atoxigenic strains from the south can be used to reduce aflatoxin production in the north. Selected non-aflatoxigenic strains specific for different agroecozones need to be identified and tested in large areas to reduce the impact of aflatoxins in maize and groundnut economies.

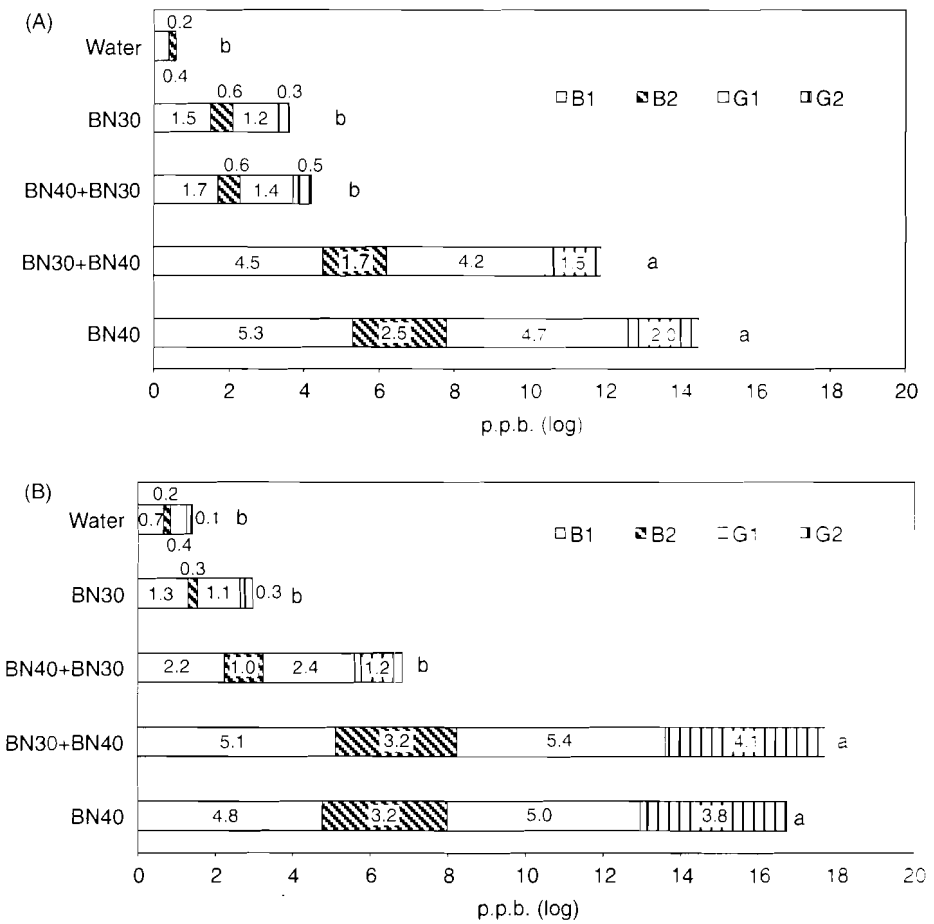

Fig. 13.1. Mean aflatoxin B1, B2, G1 and G2 in maize kernels from cobs inoculated twice at ... stage in the field with either toxigenic (BN40) or atoxigenic (BN30) strains of *Aspergillus flavus*, or one strain inoculated 7 days after another in Bénin. (A) Year 1998; 12 observations per treatment. (B) Year 1999; 24 observations per treatment. Bars not sharing a common letter are significantly different ($P < 0.001$). Log values of aflatoxins found in the samples were plotted. (Source: Cardwell and Cotty, 2000.)

Impact and Prognosis for the Future

The early beliefs that biological control agents offer more variable and less effective protection than fungicides have been refuted (Harman and Taylor, 1990). However, to achieve successful biological control, good knowledge of the host–pathogen–environment interaction is required in specific agroecosystems in which the biological control agent has to act. The interactions between microbial biological control agents, the target species to be controlled, the host and the environment can be complex and require a good research foundation prior to attempting formulation. The development of stable, cost-effective, easy-to-produce and easy-to-apply formulations of biological control agents is

another critical research step in order to achieve successful biological control of plant diseases. This is particularly true for resource-constrained situations under which agriculture is practised in Africa. Commercial use of biological control agents for plant disease control is not yet a reality in Africa, unlike the situation with biological control of insect pests that has seen spectacular successes. The final step in the development of microbial biological control agents for disease control in Africa will be to identify and define the economic and policy environment needed for successful increase and deployment of each agent. Depending on the agent, the options could be: (i) cottage industry, i.e. village/regional production as private or public enterprise; (ii) nationally organized production at central laboratories, subsidized by public funding; (iii) internationally organized production either as a one-time, donor-funded programme, or as a business enterprise picked up and exploited by existing private sector companies. For the latter to occur, it is often necessary to obtain patents for the formulation or the isolate, thereby stabilizing the proprietary status and securing the agent as a viable investment against the costs of commercialization. It is important that development of biological control options for plant diseases does not stop at the research laboratory. Commitment to development of this technology for deployment in Africa is needed at policy level, requiring that, as research laboratories enter into biological control agent testing, a conceptual framework for moving the agent to the field be part of the development agenda.

Conclusions

The development of biological control agents as a key component of integrated disease management has tremendous potential for application in the African context for the reduction of losses from plant diseases. Several biological control agents can suppress diseases as effectively as fungicides, an input that is often prohibitively expensive to be of value to resource-poor farmers. In Africa, fungal biological control agents, such as species of *Trichoderma*, *Fusarium* and *Aspergillus*, are efficacious in reducing damage caused by pathogens on maize and cowpea in research station trials. *T. koningii* and *T. harzianum* were effective in controlling damping-off of cowpea caused by *M. phaseolina*, and effective dosage and application methods have been standardized in greenhouse trials to control the disease. *F. verticillioides* is an endophytic fungus that enhances growth of maize, but becomes pathogenic to cause root and stalk rot, damping-off and ear rot when the plants undergo stress. Two strains of *T. harzianum* and *T. pseudokoningii* have been shown to reduce the stalk rot phase caused by the pathogen. These two *Trichoderma* species can penetrate the plant, move systemically within the stalk to occupy the same niche as *F. verticillioides*, and competitively exclude the pathogen. In greenhouse trials, the two species reduced stalk rot either when introduced into the stalk through injured sites or after seed treatment. Aflatoxin contamination of maize and groundnut is a serious problem in West Africa. Aflatoxins are produced by aflatoxigenic strains of *A. flavus*. Non-aflatoxigenic strains of *A. flavus* can colonize the grains, but cannot produce the mycotoxin.

Reduction in soil-borne inoculum of aflatoxigenic strains of *A. flavus* has been demonstrated after soil application with more competitive non-aflatoxigenic strains. As a result, fields receiving aflatoxigenic strain of *A. flavus* had reduced aflatoxin level in grains. These are a few examples that reveal biological control as an effective adjunct in integrated disease management. However, much more work needs to be done to demonstrate field efficacy of biological control agents, their persistence, safety and commercial feasibility, before practical application of biological control agents for plant disease control in Africa becomes a reality.

References

Abada, K.A. (1994) Fungi causing damping-off and root rot on sugar beet and their biological control with *Trichoderma harzianum*. *Agriculture, Ecosystems and Environment* 51, 333–337.

Abada, K.A. and Abdel-Aziz, M.A. (2002) Biological and chemical control of stem-canker and black-scurf on potato in Egypt. *Bulletin of Faculty of Agriculture, Cairo University* 53, 115–128.

Abdalla, M.Y. and El-Gizawy, A.M. (2000) Effect of some biological and chemical seed treatments of table beets on incidence of *Pythium aphanidermatum* damping-off in the greenhouse. *Alexandria Journal of Agricultural Research* 45, 167–177.

Adejumo, T.O., Ikotun, T. and Florini, D.A. (1999) Biological control of *Protomycopsis phaseoli*, the causal agent of leaf smut of cowpea. *Journal of Phytopathology* 147, 371–375.

Adekunle, A.T., Cardwell, K.F., Florini, D.A. and Ikotun, T. (2001) Seed treatment with *Trichoderma* species for control of damping-off of cowpea caused by *Macrophomina phaseolina*. *Biological Control Science and Technology* 11, 449–457.

Alagarsamy, G. and Sivaprakasam, K. (1988) Effect of antagonists in combination with carbendazim against *Macrophomina phaseolina* infection in cowpea. *Journal of Biological Control* 2, 123–125.

Askew, D.J. and Laing, M.D. (1994) Evaluating *Trichoderma* bio-control of *Rhizoctonia solani* in cucumbers using different application methods. *Journal of the Southern African Society for Horticultural Sciences* 4, 35–38.

Bacon, C.W., Yates, I.E., Hinton, D.M. and Meredith, F. (2001) Biological control of *Fusarium moniliforme* in maize. *Environmental Health Perspectives* 109, 325–332.

Brown, R.L., Cotty, P.J. and Cleveland, T.E. (1991) Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. *Journal of Food Protection* 54, 623–626.

Calistru, C., McLean, M. and Berjak, P. (1997) Some aspects of the biological control of seed storage fungi. In: Ellis, R.H., Black, M., Murdoch, A.J. and Hong, T.D. (eds) *Basic and Applied Aspects of Seed Biology. Proceedings of the Fifth International Workshop on Seeds*, 1995, Reading. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 755–762.

Cardwell, K.F. and Cotty, P.J. (2000) Interactions among U.S. and African *Aspergillus* spp. strains: influence on aflatoxin production. *Phytopathology* 90, 11.

Cardwell, K.F. and Cotty, P.J. (2002) Distribution of *Aspergillus* section *Flavi* among field soils from the four agroecological zones of the Republic of Bénin, West Africa. *Plant Disease* 86, 434–439.

Cherry, A.J., Jenkins, N.E., Héviefo, G., Bateman, R. and Lomer, C.J. (1999) Operational and economic analysis of a West African pilot-scale production plant for aerial conidia of *Metarhizium* spp. for use as a mycoinsecticide against locusts and grasshoppers. *Biological Control Science and Technology* 9, 35–51.

Chet, I. (1987) *Trichoderma* – application, mode of action, and potential as a biological control agent of soilborne plant pathogenic fungi. In: Chet, I. (ed.) *Innovative Approaches to Plant Disease Control*. John Wiley & Sons, New York, USA, pp. 137–160.

Cole, J.S. and Zvenyika, Z. (1988) Integrated control of *Rhizoctonia solani* and *Fusarium solani* in tobacco transplants with *Trichoderma harzianum* and triadimenol. *Plant Pathology* 37, 271–277.

Cook, R.J. (2000) Advances in plant health management in the 20th century. *Annual Review of Phytopathology* 38, 95–116.

Cook, R.J. and Baker K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society. St Paul, Minnesota, USA.

Cotty, P.J. (1994) Influence of field application of an atoxigenic strain of *Aspergillus flavus* group fungi on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. *Phytopathology* 83, 1283–1287.

Cotty, P.J. (1997) Aflatoxin-producing potential of communities of *Aspergillus* section *Flavi* from cotton producing areas in the United States. *Mycological Research* 101, 698–704.

Cotty, P.J. and Bayman, P. (1993) Competitive exclusion of a toxigenic strain of *Aspergillus flavus* by an atoxigenic strain. *Phytopathology* 83, 1283–1287.

Cotty, P.J. and Cardwell, K.F. (1999) Divergence of West African and North American communities of *Aspergillus* section *Flavi*. *Applied and Environmental Microbiology* 65, 2264–2266.

Dorner, J.W., Cole, R.J. and Blankenship, P.D. (1998) Effect of inoculum rate of biological control agents on preharvest aflatoxin contamination of peanuts. *Biological Control* 12, 171–176.

Dorner, J.W., Cole, R.J. and Wicklow, D.T. (1999) Aflatoxin reduction in corn through field application of competitive fungi. *Journal of Food Protection* 62, 650–656.

Egel, D.S., Cotty, P.J. and Elias, K.S. (1994) Relationships among isolates of *Aspergillus* sect. *flavi* that vary in aflatoxin production. *Phytopathology* 84, 906–912.

El-Zayat, M.M., Okasha, K.H.A., El-Tobshy, Z.M., El-Kohli, M.M.A. and El-Neshway, S.M. (1993) Efficiency of *Trichoderma harzianum* as a biological control agent against gray mold rot *Botrytis cinerea* of strawberry fruits. *Annals of Agricultural Science Cairo* 38, 283–290.

Emechebe, A.M. and Shoyinka, S.A. (1985) Fungal and bacterial diseases of cowpea in Africa. In: Singh, S.R. and Rachie, K.O. (eds) *Cowpea Research, Production, and Utilization*. John Wiley & Sons, Chichester, UK, pp. 173–193.

Geiser, D.M., Dorner, J.W., Horn, B.W. and Taylor, J.W. (2000) The phyllogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*. *Fungal Genetics and Biology* 31, 169–179.

Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J. and Wild, C.P. (2002) Dietary aflatoxin exposure and impaired growth in young children from Bénin and Togo: cross sectional study. *British Medical Journal* 325, 20–21.

Hall, A.J. and Wild, C.P. (1994) Epidemiology of aflatoxin-related disease. In: Eaton, D.A. and Groopman, J.D. (eds) *The Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. Academic Press, New York, USA, pp. 233–258.

Harman, G.E. and Taylor, A.G. (1990) Development of an effective biological seed

treatment system. In: Hornby, D. (ed.) *Biological Control of Soil-Borne Plant Pathogens*. CAB International, Wallingford, UK. pp. 415–426.

Hassanein, A.M., El-Barougy, E., Elgarhy, A.M., Parikka, P. and El-Sharkawy, T.A. (2000) Biological control of damping-off, root-rot/wilt diseases of alfalfa in Egypt. *Egyptian Journal of Agricultural Research* 78, 63–71.

Hell, K., Cardwell, K.F., Sétamou, M. and Poehling, H.M. (2000a) The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Bénin, West Africa. *Journal of Stored Product Research* 36, 365–382.

Hell, K., Cardwell, K.F., Sétamou, M. and Schulthess, F. (2000b) Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Bénin. *African Entomology* 8, 169–177.

Horn, B.W., Greene, R.L. and Dorner, J.W. (2000) Inhibition of aflatoxin B_1 production by *Aspergillus parasiticus* using non-aflatoxigenic strains: role of vegetative compatibility. *Biological Control* 17, 147–154.

Horn, B.W., Greene, R.L., Sorensen, R.B., Blankenship, P.D. and Dorner, J.W. (2001) Conidial movement of nontoxigenic *Aspergillus flavus* and *A. parasiticus* in groundnut fields following application to soil. *Mycopathologia* 151, 51–92.

Hutchinson, S.W. (1998) Current concepts of active defence in plants. *Annual Review of Phytopathology* 36, 59–90.

Kataria, H.R. and Sunder, S. (1985) Effect of micronutrients on the efficacy of fungicides against *Rhizoctonia solani* on cowpea seedling. *Pesticide Science* 16, 453–456.

Kerry, B.R. (2000) Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-pathogenic fungi. *Annual Review of Phytopathology* 38, 423–441.

Latunde-Dada, A.O. (1991) The use of *Trichoderma koningii* in the control of web blight disease caused by *Rhizoctonia solani* in the foliage of cowpea (*Vigna unguiculata*). *Journal of Phytopathology* 133, 247–254.

Latunde-Dada, A.O. (1993) Biological control of southern blight disease of tomato caused by *Sclerotium rolfsii* with simplified mycelial formulations of *Trichoderma koningii*. *Plant Pathology* 42, 522–529.

Leslie, J.F., Zeller, K.A. and Summerell, B.A. (2001) Icebergs and species in populations of *Fusarium*. *Physiological and Molecular Plant Pathology* 59, 107–117.

McLeod, A., Labuschagne, N. and Kotze, J.M. (1995) Evaluation of *Trichoderma* for biological control of avocado root rot in bark medium artificially infested with *Phytophthora cinnamomi*. *Yearbook of the South African Avocado Growers' Association* 18, 32–37.

McSpadden Gardener, B.B. and Fravel, D.R. (2002) Biological control of plant pathogens: research, commercialization, and application in the USA. Online *Plant Health Progress* doi:10.1094/PHP-2002-0510-01-RV. http://apsnet.org/online/feature/biological control/

Munkvold, G.P. and Desjardins, A.E. (1997) Fumonisins in maize: can we reduce their occurrence? *Plant Disease* 81, 556–565.

Navi, S.S. and Bandyopadhyay, R. (2002) Biological control of fungal plant pathogens. In: Waller, J.M., Lenné, J.M. and Waller, S.J. (eds) *Plant Pathologists' Pocketbook*. CAB International, Wallingford, UK, pp. 354–365.

Peterson, S.W., Ito, Y., Horn, B.W. and Goto, T. (2001) *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nominus*. *Mycologia* 93, 689–703.

Rahman, T.M.A., Zayed, G.A. and Asfour, H.E. (1998) Interrelationship between garlic cultivars and the biological control of white rot disease. *Assiut Journal of Agricultural Sciences* 29, 1–13.

Schulthess, F., Cardwell, K.F. and Gounou, S. (2002) The effect of endophytic *Fusarium*

verticillioides on infestation of two maize varieties by lepidopterous stemborers and coleopteran grain feeders. *Phytopathology* 92, 120–128.

Sétamou, M., Cardwell, K.F., Schulthess, F. and Hell, K. (1997) *Aspergillus flavus* infection and aflatoxin contamination of pre-harvest maize in Bénin. *Plant Disease* 81. 1323–1327.

Sétamou, M., Cardwell, K.F., Schulthess, F. and Hell, K. (1998) Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes; Monoliales) infection and alfatoxin production in maize before harvest in the Republic of Bénin. *Journal of Economic Entomology* 91, 433–438.

Sobowale, A.A. (2002) Biological control of *Fusarium moniliforme* Sheldon on maize stems by some fungal isolates from maize phyllosphere and rhizosphere. PhD thesis, University of Ibadan, Nigeria.

Triki, M.A. and Priou, S. (1997) Using chemical and biological treatments to reduce the potential of potato leak caused by *Pythium aphanidermatum* in Tunisia. *Potato Research* 40, 391–398.

Udoh, J.M., Cardwell, K.F. and Ikotun, T. (2000) Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research* 36,182–201.

Weindling, R. (1932) *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* 22, 837–845.

Wokocha, R.C. (1990) Integrated control of *Sclerotium rolfsii* infection of tomato in the Nigerian Savanna: effect of *Trichoderma viride* and some fungicides. *Crop Protection* 9, 231–234.

Yehia, A.H., Abd-El-Kader, D.A., Salem, D.E. and Sayed-Ahmed, A.A. (1994) Biological soil treatment with *Trichoderma harzianum* to control brown stem rot of soybean in Egypt. *Egyptian Journal of Phytopathology* 22, 143–157.