Research findings and strategies to reduce risks of the bioherbicide, *Myrothecium verrucaria.*

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Abstract

Studies were conducted on a *Myrothecium verrucaria* (MV) fungal isolate from sicklepod (*Senna obtusifolia* L.) that exhibits bioherbicidal activity against kudzu [*Pueraria lobata* (Willd.) Ohwi] and several other weeds. Treatments of MV plus the surfactant SilWet L-77 caused 100% mortality or control to kudzu seedlings under greenhouse conditions, and 90 to 100% control of older kudzu plants in naturally-infested and experimental kudzu plots, respectively. MV caused greater reductions of kudzu plant biomass production at 30°C, compared to 20 or 40°C in environmental chamber experiments. Responses of various non-target, young, woody plant species from several plant families to MV ranged from non-susceptible to moderately-susceptible. Bioassays of MV on seed germination and early growth of sicklepod and hemp sesbania demonstrated that hemp sesbania was more sensitive than sicklepod. Although MV possess desirable bioherbicidal traits such as high efficacy and the ability to control several species of weeds, this isolate also produces unwanted mycotoxins, i.e., trichothecenes. Data on this research is presented, as well as discussion of some future approaches to reduce or eliminate these mycotoxins in order to develop a safe and efficacious bioherbicide.

Media Summary

Myrothecium verrucaria is a fungal phytopathogen with significant bioherbicidal activity on several economically important weeds, but it also produces undesirable mycotoxins. Research data and general strategies for reducing the mycotoxin levels in this bioherbicide are presented.

Key Words

Bioherbicide, biological weed control, mycotoxin, *Myrothecium verrucaria*, trichothecenes

Introduction

Bioherbicides are phytopathogenic microorganisms or microbial phytotoxins useful for weed control. Virulence factors (lytic enzymes, phytotoxins, elicitors, etc.) for several bioherbicidal microorganisms have been elucidated, but mostly for pathogens of crop plants rather than for pathogens of weeds. In some cases phytopathogenicity has been associated with the production of compounds highly toxic to mammals. Many bioherbicidal microorganisms have also been identified with potential for weed control. Some of these microbes may produce compounds (phytotoxins and/or other metabolites) that are toxic to animals and other non-target organisms, but most have not been rigorously examined in this regard. Previous work demonstrated that an isolate of *Myrothecium verrucaria* could control sicklepod and several other herbaceous weeds (Walker and Tilley 1997). Subsequent work (Boyette et al. 2002) discovered that this isolate could control kudzu, and a U.S. patent was issued (2001) for its use as a bioherbicide for kudzu control. Recently we have attempted to develop a *Myrothecium verrucaria* (MV) isolate as a bioherbicide, but have discovered that it produces certain verrucarins (trichothecenes), which are mycotoxins. Production of such mycotoxins could hinder registration of such microbes for commercial use as bioherbicides, although, a strain of *Myrothecium verrucaria* has been registered as a nematicide, and toxicity tests of this product have proven negative on some invertebrates (Warrior 1999). A *Myrothecium verrucaria* isolate from leafy spurge has a host range (Millhollon et al. 2003; Yang and Jong 1995) that differs from that of the strain we are studying. In this paper we present some of our experimental findings on MV, plus an outline for future research to quantify and to possibly reduce or eliminate production of trichothecenes that will result in a safer bioherbicide.

Materials and Methods

Chemicals**.**

Potato dextrose agar (PDA) was purchased from Difco Laboratories, Inc., Detroit, MI. SilWet L-77 was obtained from Lovelace Industries, Greely, CO. All other chemicals used were of reagent grade purity or higher.

Myrothecium verrucaria Source and Culture.

Cultures were obtained from H.L. Walker, Lousiana Tech University, Ruston, LA. Conidial preparations of MV were produced in petri dishes containing Difco potato dextrose agar (PDA). Agar surfaces were flooded with 3 mL of a MV conidia suspension (2 x 10^6 conidia per mL). Plates were then inverted on open-mesh wire shelves and incubated (28°C, 5 da) in fluorescently lighted incubators. Conidia were rinsed from plates with sterile distilled H_2O, and adjusted with distilled H_2O to desired concentrations (estimated with hemacytometer).

Kudzu Propagation.

Kudzu seedlings were grown from seed in pots containing a 1:1 commercial potting mix/soil combination supplemented with 13:13:13 (N:P:K) fertilizer under greenhouse conditions [28 to 32°C; 40 to 60% relative humidity; 14 h photoperiod (1600 to 1800 µmol $m^{-2}s^{-1}$ PAR at midday)]. Hemp sesbania and sicklepod seedlings were grown hydroponically in paper towel cylinders (Hoagland 1995).

Kudzu Treatments.

Kudzu plants (2 to 4 leaf growth stage) were sprayed (hand-held compressed air sprayer) with inoculum (2 x 10^7 conidia per ml) until the foliage was wet (~ 500 L ha^{-1}). Silwet L-77 surfactant at 0.2% was used in all treatments, and control plants received 0.2% surfactant only. Experimental units consisted of 10 plants each and treatments were replicated three times. After inoculation, plants were placed in growth chambers at constant day/night temperatures of 20, 30, or 40°C, 12 h day/12 h night, at ~900 µmol $m^{-2}s^{-1}$ PAR. Disease development was monitored daily, and at 14 days after inoculation, both living and dead plants were excised at the soil surface, and dried (80°C, 7 da) for dry-weight determinations. A randomized complete block experimental design was utilized and means were separated using Fisher's Least Significant Difference at p = 0.05.

Host Range and Field Efficacy Tests.

Naturally forested areas. Two areas were selected in June 1999 near Yazoo City and Greenwood, MS to examine the effects of MV on various woody plant species in the field. Ten individual plants of each species (see Table1) were sprayed with MV (2.0 x 10^7 conidia per mL at 300 L per ha) in 0.2 % Silwet L-77 surfactant, or with surfactant alone. Plots (3 m x 3 m) were established in adjacent areas that were heavily infested with kudzu. These treatments were also applied to the kudzu in triplicate plots. Symptomological effects on kudzu were monitored for up to 6 weeks, and the trees were monitored on each site until late fall. A visual rating scale where: ns = not susceptible (no visual sympomatology), ss = slightly susceptible (leaf spotting and/or chlorosis), ms = moderately susceptible (leaf spots/leaf abscission at 30 to 50 %), and hs = highly susceptible (severe leaf spotting/abscission at >50 %) was utilized for the woody plants and trees. Kudzu control was determined visually as percentage control as compared to surfactant–treated plants.

Experimentally planted areas. Several species of oak (*Quercus*) trees were obtained from USDA Southern Hardwoods Forestry Research Station, Stoneville, MS and transplanted in plots in June 1999, at the SWSRU Research Farm, Stoneville, MS. Three kudzu seedlings (ca. 30 cm tall) were transplanted at the base of each tree. After acclimation (ca. 7 days) five trees of each species and the corresponding kudzu plants were sprayed with MV conidia (2 x 10^7 per mL) in Silwet L-77 (0.20 %, v/v), or with surfactant alone using a backpack sprayer at a rate of about 300 L per ha. Evaluations were made weekly over a 3-week interval.

Plant Bioassays

Seedling tests. Seeds of sicklepod and hemp sesbania were obtained from field plots at the SWSRU Research Farm, Stoneville, MS. Seeds were mechanically scarified, planted between moistened paper towels, rolled into cylinders, and grown hydroponically (Hoagland 1995) in the dark in an environmental chamber (28°C). After 4 days dark growth, uniform seedlings were selected, their shoot lengths measured

and they were placed into paper towel cylinders (5 to 8 seedlings per cylinder). Seedlings were then sprayed in triplicate with water (control), or MV conidia at several concentrations, using a hand-held compressed air sprayer. The treated seedlings were incubated at 28°C in the dark, and shoot elongation was determined at 48 and 72 h after treatment (HAT).

Seed germination and early growth tests. Sicklepod and hemp sesbania were obtained as described above. Three replicates of twenty seeds of each species were placed in wells of a 24- well-plate. Then 750:1 water:SilWet, or MV conidia at several concentrations (5 x 10^4 to 10^8 conidia per ml) with and without SilWet were added to each well. After 14 h imbibition during incubation in an environmental chamber (28°C) in the dark, the seeds were removed from each well with forceps and placed into plastic Petri dishes (60 x 15 mm) containing one filter paper disk. An additional aliquot (500 µl) of each treatment solution was added, the plates were covered, followed by incubation under the conditions described above. Seed germination and early seedling growth were recorded at several intervals (germination, 14 to 48 HAT), and growth measurements [seedling elongation (mm), 14 to 72 HAT] over a 72-h time course.

Table 1. Response of various woody plant species to *Myrothecium verrucaria*.

FAMILY Scientific name	Common name	Disease Response[1]
ANACARDIACEAE		
Rhus toxicodendron L.	Poison ivy	ms
CUPRESSACEAE		
Juniperus virginiana L.	Eastern red cedar	ns
FAGACEAE		
Quercus alba L.	White oak	ss
Q. stellata Waugh.	Post oak	ss
Q. falcata Michx.	Southern red oak	ns
Q. nigra L	Water oak	ns
Q. nuttallii Palmer	Nuttall oak	ns
Q. palustris Muench.	Pin oak	ns
Q. velutina Lam.	Black oak	ns
HAMAMELIDACEAE		
Liquidamber styraciflua L.	Sweetgum	ms
JUGLANDACEAE		
Carya aquatica (Michx. F.) Nutt.	Water hickory	ss
C. illinoisensis (Wang.) K. Koch	Pecan	ss
LAURACEAE		
Sassafras albidum (Nutt.) Nees	Sassafrass	ms
LILIACEAE		
Smilax bona-nox L.	Catbriar	ms
PINACEAE		
Pinus echinata Mill.	Shortleaf pine	ss
P. palustris Mill.	Longleaf pine	ss
P. taeda L.	Loblolly pine	ss
P. virginiana L.	Virginia pine	ss
PLATANACEAE		
Platanus occidentalis L.	American sycamore	ns
ROSACEAE		
Rubus sp.	Blackberry	ms
SALICACEAE		
Populus deltoides Marsh.	Cottonwood	ss
ULMACEAE		
Celtis laevigata Willd.	Southern hackberry	ms

[1] Visual rating scale: ns = not susceptible (no visual symptomatology), ss = slightly susceptible (leaf spotting and/or chlorosis), ms = moderately susceptible (leaf spots/leaf abscission at 30 to 50 %), and hs = highly susceptible (severe leaf spotting/abscission at >50 %).

Results and Discussion

Host range woody spp.

MV host range tests on various woody plants expected to occur in kudzu habit areas showed that over 70% of these species were not sensitive, or only slightly sensitive to MV applications (Table1). Several species were moderately sensitive, but these plants recovered from the initial injury several weeks after application.

None of the test plants exhibited mortality, or effects greater than moderately susceptible, even after fungal applications over two seasons. Original host–range tests with this isolate on a variety of mono- and dicotyledenous plants showed mortality levels > 85% for: radish, beet, chenopodium, English pea, sicklepod, hemp sesbania, and jimsonweed (Walker and Tilley 1997). Also many other species exhibited severe reductions in dry-weight accumulation, indicating broad-spectrum bioherbicidal activity. Out of 14 monocots tested, none exhibited mortality, and dry-weight reductions were low (Walker and Tilley 1997). Other studies of host range on a similar set of plants generally supported these findings (Anderson and Hallet 2004). A different isolate of *Myrothecium verrucaria* from leafy spurge (*Euphorbia esula* L.) possess a different host range on several weeds (Millhollon et al. 2003; Yang and Jong 1995).
Evaluations of kudzu control in naturally- and experimentally-infested field sites using spray applications of MV plus SilWet surfactant indicated 100 % control in the experimental plots containing transplanted seedlings, which was slightly higher than control obtained in the naturally-infested sites (Table 2). The surfactant alone caused no damage to kudzu in either of the treated plot areas.

Table 2. Biological control of kudzu in naturally- and experimentally-infested field sites

Treatment	Kudzu control (%)[a]	
	Naturally infested	Experimentally infested
MV + SilWet	88	100
SilWet alone	0	0
Untreated	0	0

[a] Values are means of three replicates.

Some isolates of MV are pathogenic to several ornamental and crop plants (Cunfer et al. 1969; Leath and Kendall 1983; Nguyen et al. 1973). Virulence and host range differences of MV isolates have also been noted (Cunfer et al. 1969; Nguyen et al. 1973; Yang and Jong 1995a). Other MV isolates have bioherbicidal activity on control thistle (*Carduus acanthoides* L.) and leafy spurge (*Euphorbia esula* L.)(Yang and Jong 1995a,b)

Kudzu tests. Kudzu was sensitive to MV when greenhouse plants (2 to 4 leaf stage) were sprayed with conidia preparations and evaluated after 5 days (Figure 1). We have also found a temperature-injury interaction of this bioherbicide on kudzu (Figure 2). Dry weight reduction in fungus-treated kudzu plants was only 40% at 20°C, but was elevated to 92 and 85% at 30°C and 40°C, respectively. This is a useful finding, since kudzu breaks dormancy and thrives during warm weather conditions.

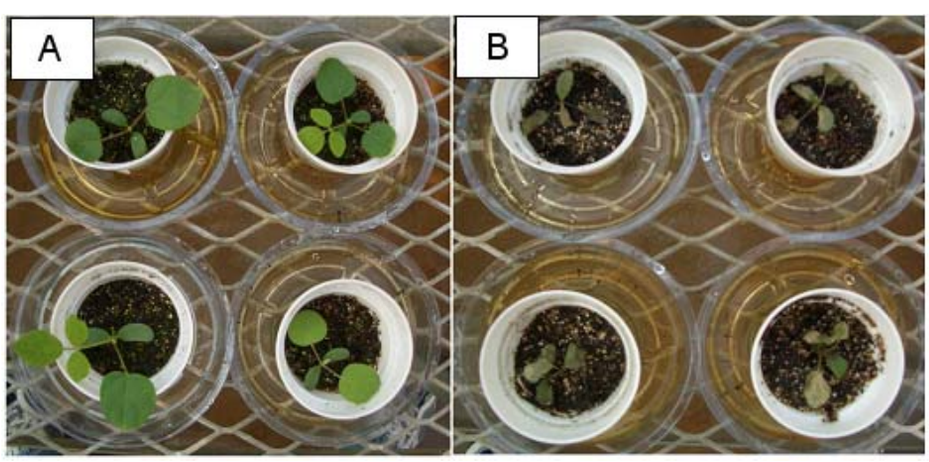

Figure 1. Effect of MV on kudzu seedlings grown under greenhouse conditions. A= untreated plants; B= plants treated with MV ($1x10^8$ conidia mL^{-1}) plus 0.2% SilWet L-77. Three days after treatment.

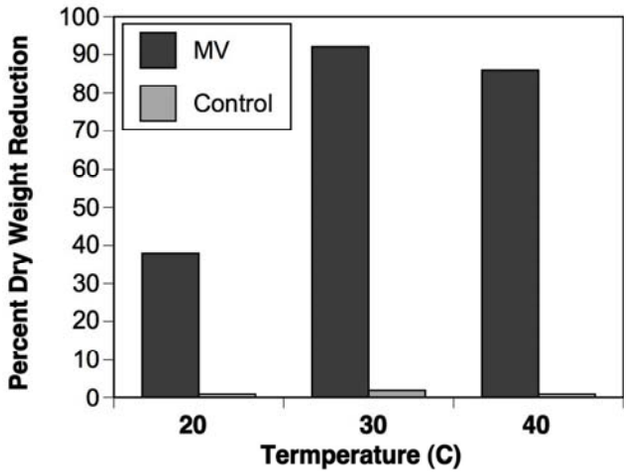

Figure 2. Effect of temperature on growth reduction of kudzu seedlings by MV conidia.

*Plant and seed bioassay tests***.** Seedling growth and mortality bioassays have been developed to evaluate bioherbicidal efficacy on sicklepod and hemp sesbania (Hoagland 1995). This bioassay system was also used to examine MV effects on young seedlings of these two species (Table 3). Seedling growth of both species was affected by relatively high conidia levels and after 24 h hemp sesbania shoot elongation was reduced by 85%, compared to 52% reduction in sicklepod. Both species were also sensitive to lower conidia concentrations, i.e., 10^6 and 10^7, and hemp sesbania growth was reduced to a greater degree (data not shown). The greater sensitivity of hemp sesbania versus sicklepod agrees with data on older plants (Walker and Tilley 1997; Anderson and Hallet 2004). This suggests the utility of this whole plant seedling bioassay for relatively rapid testing of MV, isolates and /or manipulated strains.

Table 3. Growth bioassays of *M. verrucaria* on hydroponically-grown weed seedlings.

		Shoot elongation	
Species	Treatment	48 h	96 h
Hemp sesbania	H_20	33.5a	47.2a
	MV (10^8 spores / ml)	5.2v	7.8b
	MV (3.5 x 10^8 spores / ml)	2.8c	3.4c
Sicklepod	H_20	19.7a	33.0a
	MV (10^8 spores / ml)	9.5b	16.2b
	MV (3.5 x 10^8 spores / ml)	4.2c	7.6c

Values are means of three replicates. Values followed by different letters within a column and species are significantly different (p= 0.05)

We also examined the effects of MV on seeds of these sensitive weeds. Seed germination was altered at some conidial concentrations (Figure 3). Furthermore, early growth of these two species was affected at several conidial concentrations (Figure 4). In these tests, the higher sensitivity of hemp sesbania versus sicklepod to this fungus was again demonstrated. In similar tests with kudzu seeds, germination and early growth was also drastically inhibited by MV conidia applied at similar concentrations (data not shown). Overall, results suggest that this bioherbicide might also reduce weed seed viability on mature plants, and possibly in seed banks at or near soil surfaces where the bioherbicide is applied.

Work by others on this isolate also indicates that cell-free culture filtrates contain phytotoxins (Walker and Tilley 1997; Anderson and Hallet 2004). Also in host range tests, many broadleaf species were sensitive to crude MV spray applications, but generally monocots were more tolerant.

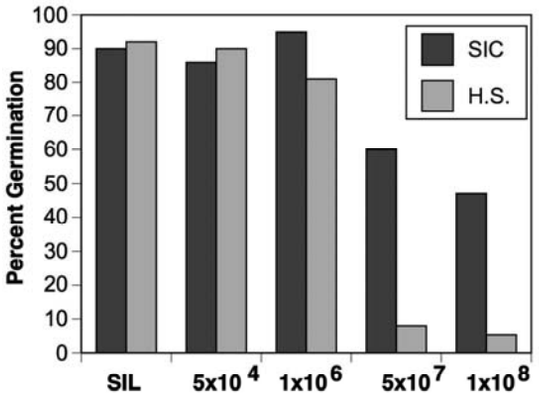

Figure 3. Seed germination of two weeds (SIC= sicklepod; H.S.= hemp sesbania) as affected by various conidial concentrations of MV. SIL = SilWet L-77 control.

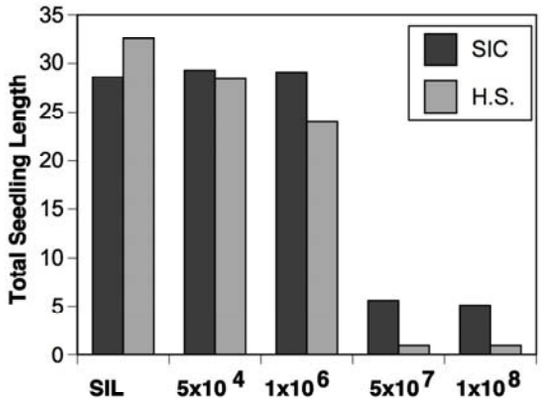

Figure 4. Seedling growth of seeds of two weeds (SIC= sicklepod; H.S.= hemp sesbania) imbibed in various conidial concentrations of MV. SIL = SilWet L-77 control.

Future Research Approaches for Safe and Effective Use of *Myrothecium verrucaria*

Cultural methods to reduce trichothecene production

Numerous conditions are known to influence the timing and amount of secondary metabolite production by fungi in culture. Altered levels of free moisture and osmotic potential (Schrodter 2004; Garrett et al. 1998; Ren et al. 1999), carbon and nitrogen levels (Abdollahi and Buchanan 1981; Kacholz and Demain 1983; Buchanan and Stahl 1984; Feng and Leonard 1998; Costa et al. 2004), temperature (Diener and Davis 1966) and pH (Jarvis 1971; Ehrlich and Cotty 2003; Keller et al. 1997) are determinants of mycotoxin production in other systems. Cultural regulation of trichothecene biosynthesis in MV is not as well studied, but similar manipulations may enable production of the bioherbicide without the risk associated with trichothecenes. We will systematically examine many of these parameters to determine their effects on trichothecene production in MV.

Chemical inhibitors to lower trichothecene production

Trichothecene synthesis is initiated from farnesyl pyrophosphate produced by farnesyl pyrophosphate synthetase (FPPS). Although many of these associated genes have been identified, few enzymes, (e.g., trichodiene synthetase (TS), and certain cytochrome P450s) have been implicated in key roles in this pathway (Trapp et al. 1998; Tag et al. 2001; Peplow et al. 2003). Various chemicals may inhibit these and other enzymes of the trichothecene pathway. Several pharmaceutical drugs are inhibitors of FPPS, i.e., nitrogen-containing bisphosphonates (zoledronic acid, minodronate, etc.) (Dunford et al. 2001), and other compounds (e.g., 1-aminoenzotriazol, piperonyl butoxide, and tetcyclasis) can inhibit cytochrome P450s. Since mevalonate is important to the pathway, mevinolin (HMG-CoA reductase inhibitor) may also alter trichothecene synthesis (Petras et al. 1999). Synthetic acid amides have been suggested to be inhibitors of P450 monooxygenases and the natural compound narengenin is also a P450 inhibitor. Cultures of MV will be exposed to potential inhibitors at various concentrations. Then, radial growth rates of MV will be measured and cultures will be assessed for trichothecene deficiency using methodology such as a *Chlorella vulgaris* bioassay (Bean et al. 1992) and/or ELISA. Trichothecene inhibitors identified in such assays, that do not reduce MV growth, will be examined for effects on the total trichothecene profile using HPLC (Abbas et al. 2001).

Selection of trichothecene deficient MV strains

Genetic and biochemical pathways leading to trichothecene production have been partially characterized in other systems (Brown et al. 2001; Kimura et al. 2003). Secondary metabolism is known to be unstable in some fungi (Bennett and Papa 1988; Horn and Doner 2001). For example, efficient commercial production of penicillin (another fungal secondary metabolite), has been made possible by numerous selections of isolates over many generations with different levels of antibiotic production (Peñalva *et al.* 1998). We have observed sector formation in MV, and noted variable levels of trichothecene production from these sectors of the same parent strain (Hoagland et al. 2005). Recurrent selection of these sectors, chemical mutagenesis, and site-directed mutagenesis may lead to further reduction or elimination of trichothecene production. The

role of trichothecenes as virulence factors on target weed species is unclear, but development of effective MV bioherbicidal strains with minimized toxicological properties may be possible.

Post-release environmental monitoring

In addition to human health risks from exposure to MV mycotoxins, environmental risks must also be considered. Host-range tests have not identified likely adverse effects on many non-target, desirable plant species, but we will monitor for spread this fungus to nearby crops and weeds. We also plan to monitor the effects of MV deployment on indigenous microflora of target weeds and associated crops though cultural independent whole-community profiling [e.g., fatty acid methyl ester (FAME) and 16S terminal restriction fragment length polymorphism (T-RFLP)]. Currently, insufficient information restricts meaningful predictions of the long-term environmental fate of MV, especially when applied augmentatively. To bridge this gap, real-time polymerase chain reaction (RT-PCR) to quantitatively monitor the deployed strain in soil and plant surfaces will be useful.

MV Risk; Constraints of product use

Greenhouse studies showed that foliar application of MV to soybean plants caused dry weight reductions of up to 75%. However, when MV was applied as a directed spray to lower stems and leaves in mixed plantings of soybean and sicklepod, soybean dry weights were not affected and sicklepod seedling mortality was 97% (Walker and Tilley 1997). Thus risks to crops may in some cases, be avoided or minimized by directed spray application of a bioherbicide.

Summary and Conclusions

Overall bioherbicides offer promising alternatives to synthetic herbicides for some applications and situations. The role of bioherbicides may be more suited to niche markets and situations where synthetic herbicides are not registered or are not developed. Bioherbicides may be used as complimentary components in successful integrated management strategies and for the discovery of new chemical classes of phytotoxins with novel modes of action. As with synthetic herbicides, there is concern of host (weed) resistance developing if a bioherbicide is used repeatedly. This is part of the risk associated with chemical or biological weed control methods. Questions regarding non-target hosts of bioherbicidal pathogens can be generally answered by extensive host range studies, however such testing is not fail-safe. There are other parallels of synthetic and biological herbicides. During developmental stages, both must be handled with minimum exposure to researchers and the environment. Another important issue in cases where a phytopathogen may produce a mycotoxin, is the determination of the active principle, and whether the phytotoxin is identical or related to the mycotoxin. As evidenced from this account, plant pathogens may contain compounds that are harmful to human health. Knowledge of the nature and regulation of production of toxic secondary compounds, and the safe handling of these agents can substantially lower or eliminate any possible health-associated risks.

References

Abbas HK, Tak H, Boyette CD, Shier WT, Jarvis BB (2001). Macrocyclic trichothecenes are undetectable in kudzu (*Pueraria montana*) plants treated with a high-producing isolate of *Myrothecium verrucaria*. *Phytochemistry* **58**, 269-276.

Abdollahi A, Buchanan RL (1981). Regulation of aflatoxin biosynthesis: induction of aflatoxin production by various carbohydrates. *Journal of Food Science* **46**, 633-635.

Anderson KI, Hallet SG (2004). Herbicidal spectrum and activity of *Myrothecium verrucaria*. *Weed Science* **52**, 623-627.

Bean GA, Jarvis BB, Aboul-Nasr MB (1992). A biological assay for the detection of *Myrothecium* spp. produced macrocyclic trichothecenes. *Mycopathologia* **119**, 175-180.

Bennett JW, Papa KE (1988). The aflatoxigenic *Aspergillus* spp. In 'Genetics of Plant Pathogenic Fungi'. pp. 263-278. (Academic Press: London).

Boyette CD, Walker HL, Abbas HK (2002). Biological control of kudzu (*Pueraria lobata*) with an isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology* **12**, 75-82.

Brown DW, McCormick SP, Alexander NJ, Proctor RH, Desjardins AE (2001). A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *F. graminearum*. *Fungal Genetics and Biology* **32**, 121-133.

Buchanan RL, Stahl HG (1984). Ability of various carbon sources to induce and support aflatoxin synthesis by *Aspergillus parasiticus*. *Journal of Food Safety* **6**, 271-279.

Costa JM, Corbellini VA, Scroferneker ML (2004). Study of different nitrogen sources on glucose uptake and production of melanin precursors and fungal mass of *Fonsecaea pedrosoi* culture in tricyclazole. *Process Biochemistry* **39**, 633-636

Cunfer BM, Graham JH, Lukezic FL (1969). Studies on the biology of *Myrothecium roridum* and *M. verrucaria* pathogenic on red clover. *Phytopathology* **59**, 1306-1309.

Diener UL, Davis ND (1966). Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopathology* **56**, 1390-1393.

Dunford JE, Thompson K, Coxon FP, Luckman SP, Hahn FM, Poulter CD, Ebetino FH, Rogers MJ (2001). Structure-activity relationships for inhibition of farnesyl diphosphate synthase *in vitro* and inhibition of bone resorption *in vivo* by nitrogen-containing bisphosphonates. *Pharmacology* **296**, 235-242.

Ehrlich K, Cotty PJ (2003). Aflatoxin control through targeting mechanisms governing aflatoxin biosynthesis in crops. *XXII Fungal Genetics Conference Proceedings* Abstr. No. 201, p 283.

Feng GH, Leonard TJ (1998). Culture conditions control expression of the genes for aflatoxin and sterigmatocystin biosynthesis in *Aspergillus parasiticus* and *A. nidulans*. *Appied and Environmental Microbiology* **64**, 2275-2277.

Garrett MH, Rayment PR, Hooper MA, Abramson MJ, Hooper BM (1998). Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. *Clinical and Experimental Allergy **28***, 459-467.

Hoagland RE (1995). Hydroponic seedling bioassay for the bioherbicides *Colletotrichum truncatum* and *Alternaria cassiae*. *Biocontrol Science and Technology* **5**, 251-259.

Hoagland RE, Weaver MA, Boyette CD (2005). ELISA detection of trichothecenes in the bioherbicide *Myrothecium verrucaria*. *Proceedings of the Weed Science Society of America* **45**, No. 213.

Horn B, Dorner JW (2001). Effect of competition and adverse culture conditions on aflatoxin production by *Aspergillus flavus* through successive generations. *Mycologia* **94**, 741-751.

Jarvis B (1971). Factors affecting the production of mycotoxins. *Journal of Applied Bacteriology* **34**, 199-213.

Kacholz T, Demain AL (1983). Nitrate repression of averufin and aflatoxin biosynthesis. *Journal of Natural Products* **46**, 499-506.

Keller NP, Nesbitt C, Sarr B, Phillips TD, Burow GB (1997). pH regulation of sterigmatocystin and aflatoxin biosynthesis in *Aspergillus* spp. *Phytopathology* **87**, 643-648.

Kimura M, Tokai T, O'Donnell K, Ward T J, Fujimura M, Hamamoto H, Shibata T, Yamaguchi I (2003). The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. *Federation of European Biochemical Societies Letters* **539**, 105-110.

Leath KT, Kendall WA (1983). Root rot alfalfa and red clover caused by *Myrothecium* spp. *Plant Disease* **67**, 1154-1155.

Millhollon RW, Berner DK, Paxson LK, Jarvis BB, Bean GW. (2003). *Myrothecium verrucaria* for control of annual morningglories in sugarcane. *Weed Technology* **17,** 276-283.

Nguyen TH, Mathur SB, Neergaard P (1973). Seed--borne species of *Myrothecium* and their pathogenic potential. *Transactions of the British Mycological Society* **61**, 347-354.

Peñalva MA, Rowlands RT, Turner G (1998). The optimization of penicillin biosynthesis in fungi. *Trends of Biotechnology* **16**, 483-489.

Peplow AW, Tag AG, Garifullina GF, Beremand MN (2003). Identification of new genes positively regulated by Tri10 and a regulatory network for trichothecene mycotoxin production. *Applied and Environmental Microbiology* **69**, 2731-2736.

Petras SF, Lindsey S, Harwood JH, Jr. (1999). HMG-CoA reductase regulation: use of structurally diverse first half-reaction squalene synthetase inhibitors to characterize the site of mevalonate-derived non-sterol regulator production in cultured IM-9 cells. *Journal of Lipid Research* **40**, 24-38.

Ren P, Ahearn DG, Crow SA (1999). Comparative study of *Aspergillus* mycotoxin production on enriched media and construction material. *Journal of Industrial Microbiology and Biotechnology.* **23**, 209-213.

Schrodter R (2004). Influence of harvest and storage conditions on trichothecene levels in various cereals. *Toxicology Letters* **153**, 47-49.

Tag AG, Garifullina GF, Peplow AW, Ake C, Jr., Philips TD, Hohn TM, Beremand MN (2001). A novel regulatory gene, Tri10 controls trichothecene toxin production and gene expression. *Applied Environmental Microbiology* **67**, 5294-5302.

Trapp SC, Hohm TM, McCormick S, Jarvis BB (1998). Characterization of the gene cluster for biosynthesis of macrocyclic trichothecenes in *Myrothecium roridum*. *Molecular and General Genetics* **257**, 421-432.

Walker HL, Tilley AM (1997). Evaluation of an isolate of *Myrothecium verrucaria* from sicklepod (*Senna obtusifolia*) as a potential mycoherbicide agent. *Biological Control* **10**, 104-112.

Warrior P, Rehberger LA, Beacu M, Grau PA, Kirfman GW, Conley JM (1999). Commercial development and introduction of DiTeraTM, a new nematicide. *Pesticide Science* **55**, 376-379.

Yang S, Jong SC (1995a). Host range determination of *Myrothecium verrucaria* isolated from leafy spurge. *Plant Disease* **79**, 994-997.

Yang S, Jong SC (1995b). Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Euphorbia esula* on species of *Euphorbia*. *Plant Disease* **79**, 998-1002.