

***Myrothecium verrucaria* fungus: A bioherbicide and strategies to reduce its non-target risks**

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ABSTRACT

Studies were conducted on a fungi *Myrothecium verrucaria* (MV) strain, originally isolated 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 °C or 40 °C, when tested in environmental chamber experiments. Responses of various non-target, young, woody plant species from several plant families to MV applications ranged from non-susceptible to moderately susceptible. Bioassays of MV on seed germination and early growth of sicklepod (*Senna obtusifolia* L.) and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. Ex A.W. Hill] demonstrated that hemp sesbania was more sensitive than sicklepod to the fungus. Although MV possesses desirable bioherbicidal traits such as high virulence and the ability to control several species of weeds, this isolate also produces undesirable mycotoxins, i.e., trichothecenes. Research data are presented, as well as some discussion of future approaches to possibly reduce or eliminate these mycotoxins to develop a safe and efficacious bioherbicide.

Key words: Bioherbicide, biological weed control, mycotoxin, *Myrothecium verrucaria*, trichothecenes.

INTRODUCTION

The study of allelopathic phenomena encompasses biochemical interactions among plants, including vascular plants and certain microorganisms in plant kingdom (38). The concept of using biotic agents as tools to suppress or to control pest populations is referred to as biological control. Bioherbicides are phytopathogenic microorganisms or microbial phytotoxins useful for biological weed control. Bioherbicides offer promising alternatives to synthetic herbicides. The role of bioherbicides may be more suited to niche markets and situations where synthetic herbicides are not registered, or are not developed, or are considered inappropriate. Bioherbicides may also be used as complimentary components in successful integrated management strategies and discover the new chemical classes of phytotoxins with novel modes of action.

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The allelopathic inhibitory interactions of phytopathogens and the phytotoxic allelochemicals from microorganisms (pathogenic and non-pathogenic) on weeds has been reviewed (7,14,15,23,25,47). 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 (49).

Generally though, existing commercialized fungal biological control agents, including bioherbicides do not pose major risks to health or the environment, because they possess host specificity and do not secrete harmful amounts of toxic and/or recalcitrant metabolites (27). But, due to the numerous bioherbicidal microorganisms that have been identified as potential agents for weed control, it is possible that some of these microbes may produce secondary metabolic compounds (phytotoxins and/or other metabolites) that are toxic to animals and other non-target organisms. Many of these microorganisms have not been rigorously examined in this regard, thus the natural chemical products they produce have not been identified, and the potential interactions or risks of the individual biocontrol microorganisms on the environment have not been accessed.

Previous work has demonstrated that a bioherbicidal isolate (IMI 361690) of *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar:Fr. (MV) can control sicklepod (*Senna obtusifolia* L.) and several other herbaceous weeds (50). Subsequent research (10) showed that this isolate could control kudzu, and a U.S. patent was issued for its use as a bioherbicide for biological control of kudzu (11). We have attempted to develop this MV isolate as a commercial bioherbicide, but one problem is that this isolate produces certain verrucarins (trichothecenes), which have potent mammalian toxicity. Such toxins produced by fungi are termed mycotoxins. The production of mycotoxins by potential bioherbicides could hinder or prevent registration of such microbes for commercial use. However, another strain of MV has been registered as a nematocide and toxicity tests of this product have proven negative on some invertebrates (51). Still another phytopathogenic MV isolate from leafy spurge (*Euphorbia esula* L.) has a host range (37,52,53) that differs from that of MV number IMI 361690, but it also produces trichothecenes. More recently, an MV isolated in Italy was shown to produce several trichothecenes (4).

This paper presents other experimental findings of MV (IMI 361690) pertaining to its host range on woody species, its efficacy on seed germination and early growth of two important weeds [hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. Ex. A.W. Hill] and sicklepod] and on its effects on greenhouse-grown and natural infestations of kudzu plants. Some discussion concerning future research to quantify and to possibly reduce or eliminate the production of trichothecenes in MV formulations, and about post-release monitoring that will result in a safer bioherbicide with reduced risks, is also outlined.

MATERIALS AND METHODS

I. Chemicals

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

II. *Myrothecium verrucaria* source and culture

Cultures of MV (IMI 361690) were obtained from H.L. Walker, Louisiana Tech University, Ruston, LA and were originally isolated from sicklepod. Conidial preparations of MV were produced in petri dishes containing PDA. Agar surfaces were flooded with 3 ml MV conidia suspension (2×10^6 conidia ml^{-1}), plates were then inverted on open-mesh wire shelves, and incubated (28 °C, 5 days) 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).

III. Plant 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 of 28 °C to 32 °C; 40 % to 60 % relative humidity and a 14 h photoperiod [$1600 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) at midday]. Seeds of sicklepod and hemp sesbania were obtained from field plots at the Southern Weed Science Research Unit Experimental Farm, at Stoneville, MS. Seeds of these two weeds were germinated and grown hydroponically in paper towel cylinders as described elsewhere (24).

IV. Kudzu plant treatments

Kudzu plants (2 to 4 leaf growth stage) were sprayed (hand-held compressed air sprayer) with inoculum (2×10^7 conidia ml^{-1}) until the foliage was completely wetted ($\sim 500 \text{ L ha}^{-1}$). 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 thrice. After inoculation, plants were placed in growth chambers at constant day/night temperatures of 20 °C, 30 °C, or 40 °C, 12 h day/12 h night, at $850 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $900 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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 days) for dry-weight determinations. A randomized complete block experimental design was used and means were separated using Fisher's Least Significant Difference at $p = 0.05$.

V. Host Range and field efficacy tests

Two natural forest areas were selected in June 1999 near Yazoo City and Greenwood, MS to examine the effects of MV on various woody plant species and on naturally established kudzu under field conditions. Ten individual plants of each species (see Table 1) were sprayed with MV (2.0×10^7 conidia ml^{-1} at 300 L ha^{-1}) 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 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 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 %) was

utilized for the woody plants and trees. Kudzu control was determined visually as percentage control as compared surfactant-treated plants.

VI. Plant bioassays

Seedling tests: Seeds of sicklepod and hemp sesbania were obtained from field plots at the SWSRU Experimental Farm, Stoneville, MS. Seeds were mechanically scarified, planted between moistened paper towels, rolled into cylinders and grown hydroponically (24) in dark in an environmental chamber (28 °C). After 4 days of dark growth, uniform seedlings were selected; their shoot lengths measured and 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 h 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-cell well-plate. Then water : Silwet (750 μ l), or MV conidia at several concentrations (5 x 10⁴ conidia ml⁻¹ to 5 x 10⁸ conidia ml⁻¹) 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 μ l) 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 hours after treatment (HAT) and growth measurements [seedling elongation (mm), 14 to 72 HAT] over a 72-h time course.

RESULTS AND DISCUSSION

I. MV woody species host range

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 (Table 1). Several species were moderately sensitive, but these plants recovered from the initial injury several weeks after treatment. The kudzu that was naturally established in close proximity to these woody species was controlled at a level of 94 % to 98 %. Similar studies on seven oak (*Quercus*) species, transplanted into transplanted kudzu plants, showed that five species were not sensitive and two were only slightly sensitive to MV applications, while the kudzu plants were 100 % controlled (28). In tests of MV on naturally infested and on experimentally infested (transplanted) kudzu plants, spray applications of MV formulated in 0.20 % Silwet L-77 provided 88 % and 100 % control of plants in the natural and experimental infestations, respectively (28). Application of the surfactant alone at this concentration caused no injury or mortality to these kudzu plants.

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

Family	Scientific name	Common name	Disease Response ¹
Anacardiaceae	<i>Rhus toxicodendron</i> L.	Poison ivy	ms
Cupressaceae	<i>Juniperus virginiana</i> L.	Eastern red cedar	ns
Hamamelidaceae	<i>Liquidamber styraciflua</i> L.	Sweet gum	ms
Juglandaceae	<i>Carya aquatica</i> (Michx. F.) Nutt.	Water hickory	ss
	<i>C. illinoisensis</i> (Wang.) K. Koch	Pecan	ss
Lauraceae	<i>Sassafras albidum</i> (Nutt.) Nees	Sassafras	ms
Liliaceae	<i>Smilax bona-nox</i> L.	Cat briar	ms
Pinaceae	<i>Pinus echinata</i> Mill.	Shortleaf pine	ss
	<i>P. palustris</i> Mill.	Longleaf pine	ss
	<i>P. taeda</i> L.	Loblolly pine	ss
	<i>P. virginiana</i> L.	Virginia pine	ss
Platanaceae	<i>Platanus occidentalis</i> L.	American sycamore	ns
Rosaceae	<i>Rubus</i> sp.	Blackberry	ms
Salicaceae	<i>Populus deltoides</i> Marsh.	Cottonwood	ss
Ulmaceae	<i>Celtis laevigata</i> 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 %). All MV applications were formulated in 0.20 % Silwet L-77.

None of the tested woody plants exhibited mortality, or effects greater than moderately sensitive, 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 (*Raphanis sativa* L.), beet (*Beta vulgaris* L.), chenopodium (*Chenopodium amaranticolor* Coste & Reynier), English pea (*Pisium sativum* L.), sicklepod, hemp sesbania, and jimsonweed (*Datura stramonium* L.) (50). Also many other species exhibited severe reductions in dry-weight accumulation, indicating broad-spectrum bioherbicidal activity for this MV isolate. Conversely, of the 14 monocots tested, none exhibited mortality, and dry-weight reductions were low (50). Other host range studies of this MV isolate on a similar set of plants generally supported these findings (3). Another isolate of MV from leafy spurge (*Euphorbia esula* L.) was found to possess a different host range when tested on several weeds (37,52,53).

Some MV isolates have been shown to be pathogenic to several ornamental and crop plants (17,36,37,39). Virulence and host range variations of different MV isolates have also been noted (17,39,52,53). Other MV isolates have bioherbicidal activity for control of thistle (*Carduus acanthoides* L.) and leafy spurge (*Euphorbia esula* L.)(52,53)

II. 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). There is also 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 92 and 85% at 30 °C and 40 °C, respectively. This is a practical finding, since kudzu breaks dormancy and thrives during warm weather conditions. Further studies on the interactions of this MV



Figure 1. Effects of MV on kudzu seedlings grown under greenhouse conditions. A= untreated plants; B= plants treated with MV (1×10^8 conidia ml^{-1}) plus 0.2% Silwet L-77. Three days after treatment.

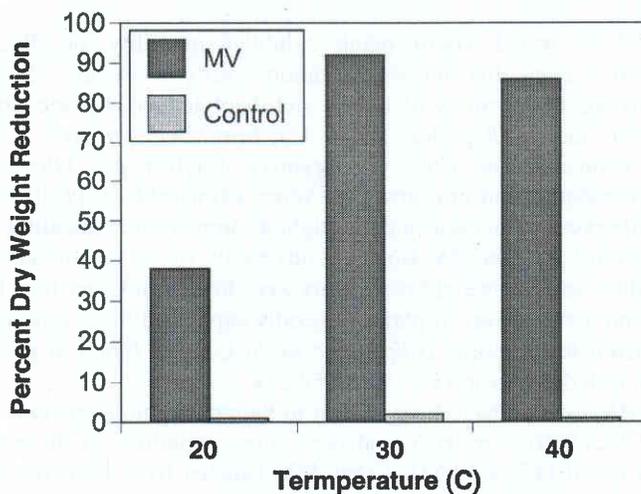


Figure 2. Effects of temperature on growth reduction of kudzu seedlings by MV conidia.

isolate with temperature and with the synthetic herbicide glyphosate have resulted in some additive and synergistic interactions of the bioherbicide and herbicide when combination treatments of these weed control agents were applied to redvine [*Brunnichia ovata* (Walt.) Shinnery], trumpet creeper [*Campsis radicans* (L.) Seem. ex. Bureau], and kudzu (9). Formulation, growth media, and application methods have also been shown to be important determinants of MV and MV sector efficacy on kudzu seedlings (26).

III. Plant and seed bioassay tests

Seedling growth and mortality bioassays have been developed to evaluate the efficacy of *Alternaria cassiae* Jurair and Khan and *Colletotrichum truncatum* (Schwein.) Andrus and W.D. Moore, which are bioherbicides for sicklepod and hemp sesbania, respectively (24). This bioassay system was also used to examine MV effects on young seedlings of these two species (Table 2). 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 conidia ml⁻¹, and hemp sesbania growth was reduced to a greater degree (data not shown). The greater sensitivity of hemp sesbania versus sicklepod is in agreement with other published data on older plants (3,50). These findings suggest the utility of using this whole plant seedling bioassay for relatively rapid testing of MV, isolates and /or manipulated strains.

Table 2. Growth bioassays of *M. verrucaria* on hydroponically-grown weed seedlings

Weed species	Treatment	Shoot elongation (mm)	
		48 h	96 h
Hemp sesbania	Water	33.5a	47.2a
	MV (10^8 spores ml ⁻¹)	5.2b	7.8b
	MV (3.5×10^8 spores ml ⁻¹)	2.8c	3.4c
Sicklepod	Water	19.7a	33.0a
	MV (10^8 spores ml ⁻¹)	9.5b	16.2b
	MV (3.5×10^8 spores ml ⁻¹)	4.2c	7.6c

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

The effects of MV on seeds of these sensitive weeds were also investigated. 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 demonstrated again. In similar tests with kudzu seeds, germination and early growth was also drastically inhibited by MV conidia applied at similar concentrations (data not shown). Recently MV was shown to be highly virulent against several serious weeds of commercial tomato (*Lycopersicon esculentum* L.) fields in the southeastern U.S. (8). The weeds were treated with MV several days prior to the transplantation of tomato seedlings, after which the transplanted tomato plants grew and remained vigorous and healthy through the growing season. Using MV in this manner suggests that MV may have potential as a pre-plant bioherbicide in production systems such as transplanted tomato. Work by others on this isolate also suggests that cell-free culture filtrates contain phytotoxins (3,50). Also, in host range tests, many broadleaf species were sensitive to crude MV spray applications, but generally monocots were more tolerant. Overall, these and the above results suggest that this bioherbicide might reduce weed seed viability on mature plants, and possibly in seed banks at or near soil surfaces where the bioherbicide is applied. Trichothecenes produced by an MV isolate from Italy could inhibit seed germination of the parasitic plant, *Orobancha ramosa* (4). This indicates that part of the phytotoxicity of this newly discovered MV isolate may be due to the action of trichothecenes.

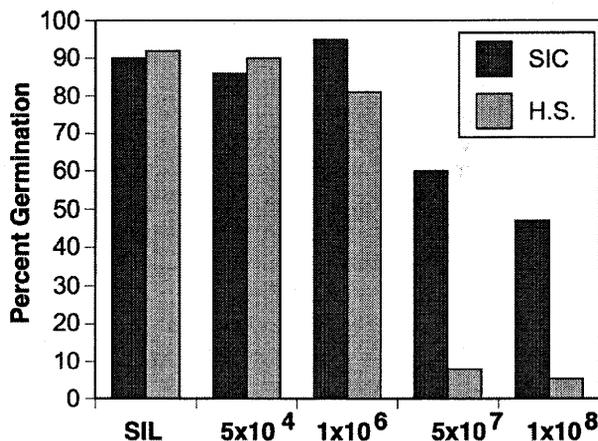


Figure 3. Seed germination of two weeds (SIC = sicklepod; H.S. = hemp sesbania) as affected by various conidial concentrations of MV. SIL = Silwet L-77 control.

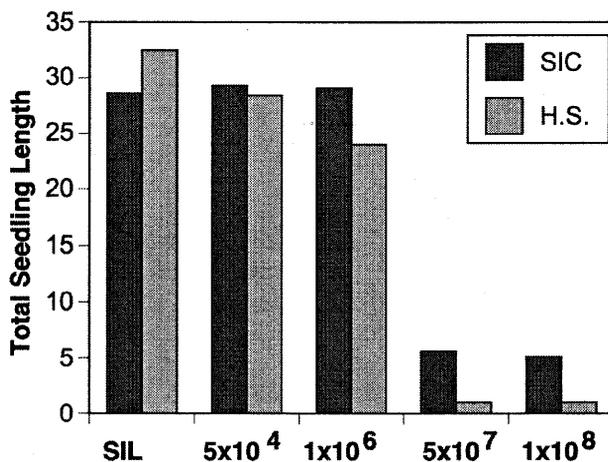


Figure 4. Seedling growth of seeds of two weeds (SIC = sicklepod; H.S. = hemp sesbania) imbibed in various conidial concentrations of MV. SIL = Silwet L-77 control.

IV. Future research approaches for safe and effective use of *Myrothecium verrucaria*

(i). **Cultural methods to reduce trichothecene production** : Numerous parameters are known to influence the biosynthesis and amount of secondary metabolite production by fungi in culture. Altered levels of free moisture and osmotic potential (22,44,45), carbon and nitrogen levels (2,13,16,21,32), temperature (18) and pH (20,31,33) have been shown to be determinants of mycotoxin production in other systems. Although cultural regulation of trichothecene biosynthesis in MV is not as well studied or understood, similar

manipulations may enable production of this bioherbicide without the risk associated with trichothecenes. In future research, a systematic examination of many of these parameters will determine their effects on trichothecene production in this bioherbicide. As indicated above, formulation, growth media, and application methods can influence MV and MV sector efficacy on kudzu seedlings (26), and thus virulence factors, phytotoxins, and perhaps mycotoxin production may have been altered via the experimental parameters imposed on these MV and MV sector cultures. Further research will be necessary to ascertain this.

(ii). Chemical inhibitors to lower trichothecene production: Trichothecene synthesis is initiated from farnesyl pyrophosphate produced by farnesyl pyrophosphate synthetase (FPPS). Although many of the genes associated with this biosynthetic pathway have been identified, only a few enzymes, (e.g., trichodiene synthetase (TS), and certain cytochrome P450s) have been implicated to play key roles in this pathway (41,46,48). 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.) (19), 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 (42). Synthetic acid amides have been suggested to be inhibitors of P450 monooxygenases and the natural compound narengenin is also a P450 inhibitor. Natural products in cell wall extracts from a susceptible muskmelon (*Cucumis melo* L.) cultivar significantly increased trichothecene production by *Myrothecium roridum* (pathogenic to muskmelon), while extracts from a resistant cultivar inhibited mycotoxin production (35). To discover compounds useful as regulators of trichothecene levels *in vivo*, 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 (5) and/or ELISA (29). Trichothecene inhibitors identified in such assays, that do not compromise MV growth, will be examined for effects on the total trichothecene profile using HPLC (1).

(iii). Selection of trichothecene deficient MV strains : Genetic and biochemical pathways leading to trichothecene production have been partially characterized in other systems (12,34). Secondary metabolism is known to be unstable in some fungi (6, 30). 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 (40). The formation of sectors in MV cultures, and noted variable levels of trichothecene production from these sectors as measured by ELISA (28) suggests alteration or changes in the efficiency of this mycotoxin pathway. Recurrent selection of these sectors, chemical mutagenesis, and site-directed mutagenesis may lead to further reduction or elimination of trichothecene production. Although trichothecenes are thought by some researchers to be virulence factors in some *Fusarium* phytopathogens (43), the role of these mycotoxins as virulence factors on MV target weed species is unclear. Nevertheless, the development of effective MV bioherbicidal strains with minimized toxicological properties may be possible.

(iv). **Post-release environmental monitoring** : In addition to human health risks from exposure to MV mycotoxins, environmental risks should also be considered. Host-range tests have not identified likely adverse effects on many non-target, desirable plant species, but plans are underway to monitor for spread this fungus from application sites to nearby crops and weeds. Protocols to monitor the effects of MV deployment on indigenous microflora of target weeds and associated crops through cultural independent whole-community profiling [e.g., fatty acid methyl ester (FAME) and 16S terminal restriction fragment length polymorphism (T-RFLP)] have also been developed. 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 on plant surfaces will be useful.

MV Risk: Constraints of product use

Greenhouse studies revealed 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 % (50). Thus risks to crops may in some cases, be avoided or minimized by using rather simple approaches such as directed spray application of a bioherbicide. This is analogous to the application of some synthetic herbicides.

CONCLUSIONS

Myrothecium verrucaria is a unique biological control agent. It has been shown to kill a broad spectrum of weeds using post-emergence application, but it also exhibits some pre-emergence activity. This phytopathogen also requires a unique risk management strategy. Because of its broad spectrum activity a pro-active application system to avoid damage to non-target vegetation will be required. Questions regarding non-target hosts of bioherbicidal pathogens can be generally answered by extensive host range studies, however such testing is not fail-safe. Another significant risk factor that must be managed is the toxicity of secondary metabolites produced by MV. Secondary metabolites of other fungi have been manipulated by alterations of the cultural conditions, and a similar approach may be effective for MV. Alternatively, chemical inhibitors or toxin-free strains of MV might be identified.

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 either chemical or biological weed control methods. There are also other parallels of synthetic and biological herbicides. During developmental stages, both must be handled with minimum exposure to researchers and the environment. Other important issues in cases where a phytopathogen may produce a mycotoxin, are identification of the active principle, and ascertaining whether the phytotoxin is identical to, or related to the mycotoxin. As pointed out here, plant pathogens may contain compounds that are harmful to human health. Knowledge of the identity and production of toxic secondary compounds,

and the safe handling of these agents can substantially lower or eliminate any possible health-associated risks. Through identification of the nature and magnitude of the risks associated with MV, it may be possible to develop MV as a safe and effective bioherbicide.

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