

# Developmental Studies of *Myrothecium verrucaria* (IMI 361690) as a Bioherbicide

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## ABSTRACT

We are presently developing *Myrothecium verrucaria* (MV; strain IMI 361690), first isolated from the weed sicklepod, as a bioherbicide for kudzu and other invasive weeds. Kudzu is our initial primary target weed since it is very difficult to control, and is also a host for Asian soybean rust. Our studies showed that MV produced mycotoxins (macrocyclic trichothecenes), and this factor has impeded its acceptance and approval for registration by EPA. Over the past few years, we have examined various aspects to advance the utility and acceptability of MV as a commercial product. We have discovered that:

- MV is generally weakly pathogenic, but is highly efficacious against several weeds when combined with the surfactant Silwet L-77;
- MV can control diverse dicotyledonous weeds (kudzu, hemp sesbania, redivine, trumpet creeper, morningglories, pigweeds, etc.);
- MV interacts additively and synergistically with the herbicide glyphosate to provide more efficacious weed control;
- MV produces several trichothecenes; we have developed methodologies (HPLC and ELISA) to detect & quantify these mycotoxins; and
- production of these mycotoxins can be mitigated using mycelial formulations grown in sub-merged culture, washed spore formulations, or by modifying nutritional components whilst high bioherbicidal activity is maintained.

Current investigations include: the bioherbicidal activity of various chemically-induced mutants of MV, the role of hydrolytic enzymes in MV pathogenicity, the ultra-structural effects of MV action on kudzu and other weed tissues, and the use of biomarkers to monitor infection process, over-wintering, and ecological impacts of MV applications in the environment. Understanding the bioherbicidal mechanisms of action and the elimination of mycotoxins in formulations will help promote MV as a safe and effective bioherbicide product.

## INTRODUCTION

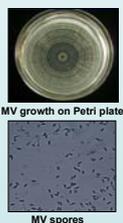
Kudzu [*Pueraria lobata* (Willd.) Ohwi], a perennial leguminous vine native to eastern Asia, was introduced into the U. S. in the late 1800's, (McKee & Stephens, 1943) and now occurs from Florida to New York, westward to central Oklahoma and Texas, with the heaviest infestations in Alabama, Georgia, and Mississippi (Miller, 1997).



Natural infestations of kudzu.

Cited in a 1993 Congressional Report as one of the most harmful non-indigenous plants in the U. S., kudzu became a federal noxious weed in 1998. This aggressive weed is very difficult to control using synthetic chemical herbicides, and has recently been identified as an over-wintering host of Asian soybean rust (*Phakopsora pachyrhizi*).

Bioherbicides (microorganisms and/or their products) can cause injury and/or mortality to weeds. The phytopathogenic fungus *Myrothecium verrucaria* (MV) (Alb. & Schwein.) Ditmar.Fr., (IMI 361690), originally isolated from the weed sicklepod (*Senna obtusifolia* L.), has high bioherbicidal activity, and exhibits excellent biological control of several weed species [sicklepod, hemp sesbania (*Sesbania exaltata*), etc.] when formulated with Silwet L-77 (silicone-polyether surfactant) (Walker and Tilley, 1997). A patent for use of this fungus as a biological weed control agent was issued (Boyette et al. 2001). A negative factor regarding MV is the risk associated with its production of mycotoxins (trichothecenes) (Hoagland et al. 2008a).



MV growth on Petri plate

MV spores

## TESTS of MV HOST RANGE and INTERACTIONS with GLYPHOSATE

We have demonstrated high MV efficacy on several weeds including kudzu, various morning-glory species, redivine, and trumpet creeper (Boyette et al. 2000; Boyette 2002; Boyette et al. 2008a; Hoagland et al. 2007a, 2007b; Hoagland et al. 2008a, 2008c). Several other weeds (purslanes and spurge) were also controlled with MV allowing transplantation of tomato seedlings without crop injury (Boyette et al. 2007).

MV effectively controls kudzu in the absence of a dew treatment over a wide range of physical, environmental, and field conditions. Reports indicate significant performance improvements of several bioherbicides through additive or synergistic effects of chemicals (Hoagland, 1996).



Kudzu controlled with MV  
Untreated (left), MV-treated (right).

MV effects on morning-glory species.

A = Untreated  
B = invert emulsion  
C = MV  
D = MV + invert

We have shown synergistic interactions of MV and glyphosate on kudzu and some other invasive weeds (Boyette et al. 2006; Boyette & Hoagland, 2000; Boyette et al. 2008a).



Effect of MV and glyphosate on kudzu.

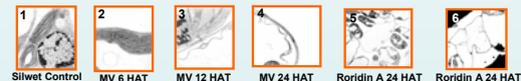
A = untreated; B = gly;  
C = MV; D = gly + MV.

Natural mixed infestation of redivine and trumpet creeper controlled by MV + glyphosate.

MV host range tests on tree species commonly found in natural kudzu infestations indicated most trees were not susceptible to MV (Hoagland et al. 2005).

## ULTRASTRUCTURAL INVESTIGATIONS

- MV effects on kudzu seedling leaflets (Boyette et al. 2008b.) revealed;
- >No visual or ultra-structural effects occurred with MV spores or surfactant alone (Plate 1)
  - >Rapid (~ 1-6 HAT) protoplast detachment from cell walls (Plate 2)
  - >Plasmodesmata broken off and retained in cell walls at 12 HAT (Plate 3)
  - >Ultra-structural symptoms occurred prior to fungal growth structures (Plate 4)
  - >Fungal growth observed following severe tissue degeneration (24-48 HAT), but growth primarily on leaf surfaces
  - >Roridin A (trichothecene mycotoxin, produced by unwashed MV spores, but not washed spores or mycelium) caused symptoms similar to those induced by spores plus surfactant (Plates 5 & 6)
  - >Data indicate penetration of phytotoxic substance(s) in fungal formulation plus surfactant, and that Roridin A has phytotoxic properties similar to MV toxin(s)
  - >Overall effects appears unique among known phytotoxins or mycotoxins



Silwet Control MV 6 HAT MV 12 HAT MV 24 HAT Roridin A 24 HAT Roridin A 24 HAT

## MYCOTOXIN QUANTIFICATION and ELIMINATION

We developed methods to detect and quantify trichothecenes produced by MV (Boyette et al. 2008c; Hoagland et al. 2008b).



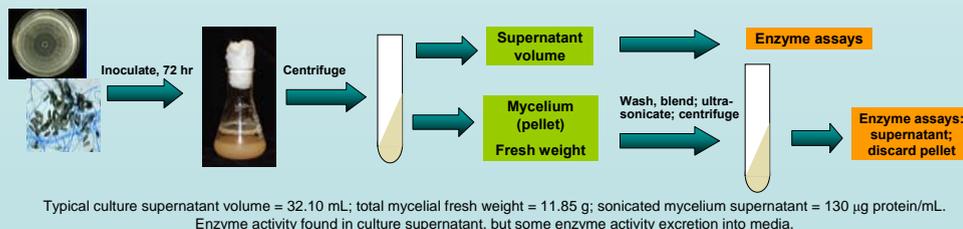
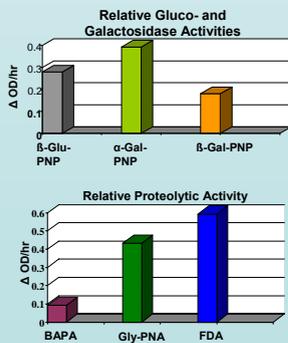
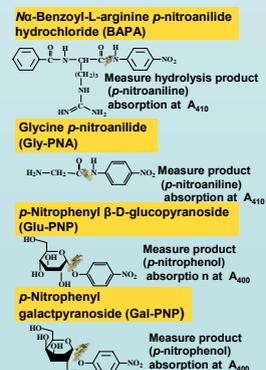
HPLC chromatogram of trichothecene profile in un-washed MV spores (left), washed MV spores or mycelium (right).

Elisa standard curve of roridin A, a trichothecene from MV.

We also discovered methods to reduce or eliminate trichothecenes via washing MV spores (Weaver et al. 2007), and by using mycelium (produced via fermentation) as inoculum (Boyette et al. 2008c).

## VIROLENCE FACTOR STUDIES--ENZYMOLGY

The role of cell wall degrading enzymes in infection processes of phytopathogenic microorganisms is well documented. Most attention has focused on hydrolytic enzymes that degrade polysaccharides, but recently lytic enzymes such as proteases have been implicated as virulence factors in certain host plant:phytopathogen interactions.



## CONCLUSIONS

- >Washed MV spores or using MV mycelium as inoculum provides high efficacy and eliminates mycotoxins.
- >Cytological investigations at the ultra-structural level will yield valuable information on the mechanism of action of MV.
- >Knowledge of MV virulence factors is crucial to aid the development and improvement of a commercial product.
- >Little known about biochemical processes involved during infection and the onset of necrosis in MV treated weeds.
- > Knowledge of such events and their biochemical mechanisms will be useful to regulate and/or improve bioherbicidal efficacy.

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