Interactions of quinclorac with a bioherbicidal strain of *Myrothecium verrucaria*

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ABSTRACT

- We are developing the fungus, *Myrothecium verrucaria* (MV) as a bioherbicide for kudzu and several other weeds.
- Spore and mycelial MV formulations cause rapid injury/mortality to these weeds; some herbicides act additively or synergistically with MV.
- Ultra-structurally MV causes protoplast detachment from the cell wall in kudzu, accompanied by broken-off plasmodesmata retained in cell walls.
- Bioassays/greenhouse tests show the herbicide quinclorac acts additively and/or syngeristically with MV on sicklepod, hemp sesbania and kudzu.
- Research on quinclorac/MV interactions (*in planta*, ultra-strurcturally, field conditions) is on-going to characterize roles in infectivity, necrosis and death of target weeds.

INTRODUCTION

Bioherbicides are microorganisms that can cause injury and/or mortality to weeds. The phytopathogenic fungus *Myrothecium verrucaria* (MV) (Alb. & Schwein.) Ditmar:Fr. (strain IMI 361690), has high bioherbicidal activity and controls several weeds when formulated with Silwet L-77 (silicone-polyether surfactant) (Walker and Tilley, 1997). A U.S. patent for use of this fungus as a biological weed control agent was issued (Boyette et al. 2001) and research has demonstrated its efficacy on various weeds (e.g., Boyette et al. 2006; Boyette et al. 2007; Hoagland et al. 2007). We are attempting to develop this organism as a bioherbicide, especially for control of kudzu [*Pueraria lobata* (Willd.) Ohwi].

Kudzu | Sicklepod | Hemp sesbania

Kudzu, a perennial leguminous vine native to eastern Asia, was introduced into the U. S. in the late 1800's, and now occurs from Florida to New York, westward to central Oklahoma and Texas, with heavy infestations in Alabama, Georgia, and Mississippi (Miller, 1997). In 1993 a Congressional Report cited it as one of the most harmful non-indigenous plants in the U. S. This aggressive weed is difficult to control using synthetic chemical herbicides, and is an over-wintering host of Asian soybean rust (*Phakopsora pachyrhizi*).

Sicklepod (*Senna obtusifolia* L.), the host weed from whence MV (IMI 361690) was isolated, is an annual herbaceous/semi-woody legume that occurs throughout southeastern U. S. It may reach heights of 6 ft, is a pest in cotton, corn, and soybeans, can reduce cotton yield ~3% per weed per 30 row-ft), and is one of the world's worst weeds (Holm et al. 1997).

Hemp sesbania (*Sesbania exaltata*), a leguminous weed in soybean, cotton, and rice can attain heights of ~10 ft, and is one of 10 most troublesome weeds in Arkansas, Louisiana, and Mississippi (Dowler 1997). It produces numerous seeds (≤ 21,000 seeds/plant) (Norsworthy & Oliver 2002), and is very competitive with crops. MV provides excellent control of hemp sesbania.

MV controls kudzu in the absence of dew over a wide range of physical, environmental, and field conditions. However, enhanced performance of some bioherbicides through additive or synergistic effects of chemicals, especially with the herbicide glyphosate have been reported (Boyette & Hoagland, 2000; Hoagland, 1996). We have shown synergistic interactions of MV and glyphosate on kudzu and some other invasive weeds (Boyette et al. 2006).

Kudzu controlled in a natural infestation with *Myrothecium verrucaria* (MV); untreated (left), MV-treated (right).

Recently, the bioherbicidal activity of a fungal pathogen (*Pyricularia setariae*) of the weed green foxtail (*Setaria viridis*) was found to be synergized by several herbicides, including quinclorac (Peng & Byer 2005). Several synthetic auxin-type herbicides are labeled for kudzu control. The auxin-type herbicide quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) provides excellent control of some grasses and broadleaf weeds but it is not labeled for kudzu. Quinclorac is highly selective, but the basis for selectivity remains obscure, and its molecular mode of action is controversial.

To aid in the development/improvement of a commercial product of MV, knowledge of its virulence factors and additive, antagonistic, or synergistic interactions with other agochemicals is crucial.

OBJECTIVES

To examine the effects of quinclorac alone and in combination with MV on hemp sesbania and sicklepod using bioassays and in greenhouse-grown kudzu seedlings.

MATERIALS and METHODS

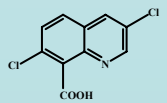
Inoculum production. MV spores [*M. verrucaria* (IMI 361690)] cultured in petri dishes on potato dextrose agar (PDA); incubated (25 ° C, 5 da); conidia (spores) harvested with sterile H_2O; concentration determined (hemacytometer). Inoculate fermenter with spores; grow on proprietary liquid medium (48-72 hr); determine mycelial concentration (fresh weight).

Bioassays: kudzu, sicklepod, hemp sesbania

Bioassay. Bioassays utilized 4-day old, dark-grown (hydroponically) sicklepod or hemp sesbania seedlings. Five excised shoot segments (upper 10 mm) containing cotyledons, placed in petri dishes containing water (control), MV, quinclorac , or MV + quinclorac. Treatments placed in light chamber; analyzed [elongation, fresh weight and chlorophyll accumulation (Hiscox & Israelstam 1979)] after 72 hr.

Greenhouse tests. Kudzu seeds were planted and grown in a potting soil mixture; 2-week-old plants were treated. All treatments contained 0.1% Silwet (v/v) applied using hand-held sprayers; data taken 7 days after treatment (DAT).

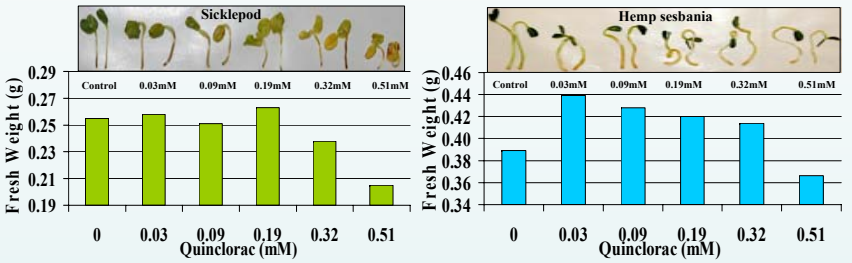
Quinclorac. Quinclorac (auxin-type herbicide, purity = 98%) obtained from Chem Service, West Chester, PA.



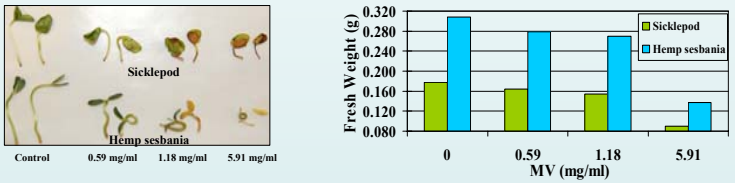
Quinclorac
3,7-dichloro-8-quinolinecarboxylic acid

RESULTS

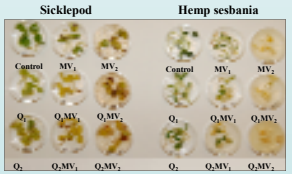
Effects of Quinclorac Concentration on Excised Sicklepod and Hemp Sesbania Seedling Shoot Segments



Effects of MV Mycelial Concentration on Excised Sicklepod and Hemp Sesbania Seedling Shoot Segments

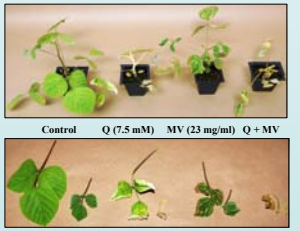


Effects of MV, Quinclorac, and MV plus Quinclorac on Excised Sicklepod and Hemp Sesbania Seedling Shoot Segments



Control = H_2O; MV_1 = 0.89 mg/ml; MV_2 = 3.6 mg/ml; Q_1 = 0.09 mM; Q_2 = 0.51 mM

Effects of Spray Applications of MV, Quinclorac, and MV plus Quinclorac on Intact Kudzu Seedlings, 7DAT



SUMMARY

- Quinclorac alone caused swelling in sicklepod and hemp sesbania at low concentrations, but effect was subjugated at higher levels where growth and chlorophyll accumulation were drastically inhibited
- MV alone significantly inhibited growth and chlorophyll accumulation (with only slight or no swelling); directly proportional to increased concentration
- Low concentrations of MV + quinclorac induced swelling in sicklepod, but not hemp sesbania; no swelling in sicklepod as MV or quinclorac concentrations increased; combinations of MV and quinclorac were additive and/or synergistic
- Combinations of MV + quinclorac prevented severe swelling in kudzu (caused by quinclorac alone), and exhibited additive and/or synergistic injury/mortality effects
- Ultra-structural analysis of MV and quinclorac interactions in these tissues is in progress
- Chlorophyll extraction/quantification and growth (data not shown) corroborated the visual injury exhibited by treatments
- Further characterization of these findings are being investigated/extended to greenhouse and field studies

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