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Interactions of Quinclorac with a Bioherbicidal Strain of *Myrothecium verrucaria*

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ABSTRACT

The fungus, *Myrothecium verrucaria* (Alb. & Schwein.) (IMI Accession No. 3601690) (MV), is being developed as a bioherbicide for kudzu [*Pueraria lobata* (Willd.) Ohwi] and other invasive weeds. Spore and mycelial formulations of MV exhibit relatively rapid bioherbicidal activity when applied to the foliage of these weeds, and that application of MV with the herbicide glyphosate [*N*-(phosphonomethyl)glycine] can exhibit synergistic herbicidal interactions in certain instances. Several synthetic auxin-type herbicides are labeled for use to control kudzu. The auxin-type herbicide quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) is not labeled for kudzu control, but is effective on hemp sesbania [*Sesbania exaltata* (Raf.) Rybd. Ex. Hill]. In bioassays of hemp sesbania and sicklepod (*Senna obtusifolia* L.) seedlings and in greenhouse tests using kudzu plants, sub-lethal concentrations of both MV and quinclorac (high purity, technical grade) applied to plant tissues caused some additive and/or synergistic effects on the reduction of growth and chlorophyll accumulation. These important findings under controlled conditions provide the basis for further characterization of MV and quinclorac interactions on weeds under field conditions.

Keywords: bioherbicide, biological weed control, fungus, herbicide, interaction, phytopathogen
Abbreviations: DAT, days after treatment; **MV**, *Myrothecium verrucaria*; **PPF**, photosynthetic photon flux

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 bioherbicidal activity and can control several weeds when formulated with Silwet L-77 (silicone-polyether non-ionic 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, 2007; Hoagland *et al.* 2007). Presently, we are attempting to develop this organism as a bioherbicide, especially for control of kudzu [*Pueraria lobata* (Willd.) Ohwi].

Kudzu, a perennial leguminous vine native to eastern Asia, was introduced into the U.S. in the late 1800's, (McKee and Stephens 1943) and now occurs from Florida to New York, westward to central Oklahoma and Texas, with heavy infestations in Alabama, Georgia, and Mississippi (Miller 1996). In 1993, a Congressional Report cited kudzu as one of the most harmful non-indigenous plants in the U.S. (Anonymous 1993). 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* (Syd. & P. Syd.) (Jurick *et al.* 2008).

Sicklepod (*Senna obtusifolia* L.) is the host weed from which MV (IMI 361690) was isolated. Sicklepod is an annual, herbaceous/semi-woody legume, native to the American tropics and occurs throughout southeastern U.S. It may reach heights of 2 m, and is a pest in cotton, corn, and soybeans. This weed can reduce cotton yield by ~3% for each weed occurring in a 9 m row (Steckel 2006), and is classified as 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 over 3 m (Lorenzi and Jeffery 1987). It is one of 10 most troublesome weeds in Arkansas, Louisiana, and Mississippi (Dowler 1997), reducing crop yield by shading and competition (Bryson 1987; Bryson 1990; King and Purcell 1997). Hemp sesbania produces numerous seeds (≤ 21,000 seeds per plant) (Norsworthy and Oliver 2000), and at densities of 0.8–12.9 plants m^{-2} over a season, can reduce soybean yield up to 80% (McWhorter and Anderson 1979). MV provides excellent control of hemp sesbania (Walker and Tilley 1997).

An important consideration for herbicides and for bioherbicides, is to retain high efficacy over a variety of conditions. MV has been shown to control kudzu in the absence of dew over a wide range of physical, environmental, and field conditions (Boyette *et al.* 2002). Furthermore, enhanced performance of some bioherbicides through combined application or formulation with chemicals can provide additive or synergistic effects, especially the herbicide glyphosate (Hoagland 1996; Boyette and Hoagland 2000). Previous research has shown synergistic interactions of MV and the herbicide glyphosate on kudzu and some other invasive weeds (Boyette *et al.* 2006). Various combinations of glyphosate and another bioherbicidal fungus, *Colletotrichum truncatum* (Schwein.) Andrus & Moore, were also found to cause additive or synergistic interactions and improved weed control of hemp sesbania (Boyette *et al*. 2008b).

Recently, the bioherbicidal activity of a pathogen (*Pyricularia setariae* Nisikado) of the weed green foxtail (*Setaria viridis* L.) was found to be synergized by several herbicides, including the auxin-type herbicide, quinclorac (Peng and Byer 2005). Several synthetic auxin-type herbicides are labeled for kudzu control. Quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) provides excellent control of some grasses and broadleaf weeds, but it is not labeled for kudzu control. Quinclorac provides fair to good control of hemp sesbania in rice (Anonymous 2005). Quinclorac is highly

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selective, but the basis for its selectivity remains obscure and its molecular mode of action is not well understood.

Quinclorac is a highly selective auxinic herbicide is widely used in rice (*Oryza sativa* L.) primarily for the control of *Echinochloa* spp. and certain dicot weeds including *Sesbania* spp (Grossmann 1998). This herbicide was originally thought to act as a synthetic auxin (Berghaus and Wuerzer 1987). However, while quinclorac-susceptible broad–leaf plants demonstrated auxin-like symptoms (Berghaus and Wuerzer 1987; Koo *et al*. 1991), quinclorac-susceptible grasses exhibited symptoms different than those caused by 2,4-D (2,4-dichlorophenoxyacetic acid) (Berghaus and Wuerzer 1987; Koo *et al.* 1991; Sunohara and Matsumoto 1997). Many studies of quinclorac effects on grass species showed that quinclorac elevated ethylene production via stimulating 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, and the levels of endogenous ACC leading to high levels of cyanide. Thus, quinclorac-stimulated cyanide production was suggested to cause chlorosis in grasses (Grossmann and Kwiatkowski 1995) and this was suggested as the primary mechanism of action in grasses (Grossmann and Kwiatkowski 2000). Recent support of this mechanism in another grass species, smooth crabgrass (*Digitaria ischaemum*) has been reported (Abdallah *et al.* 2006). Generally, the differential activity of quinclorac on grasses and broad-leaf plants distinguishes it from most other auxenic herbicides.

To aid in the development and improvement of MV as a commercial product, knowledge of its virulence factors and additive, antagonistic, or synergistic interactions with other chemicals or agochemicals as described by Hatzios and Penner (1985) is crucial. The objective of this research is to investigate the effects of quinclorac alone and in combination with MV on three important leguminous weeds (hemp sesbania, sicklepod and kudzu) with the hope of discovering important interactions of the herbicide and bioherbicide. Laboratory bioassays were performed on hemp sesbania and sicklepod and tests on kudzu seedlings were carried out in the greenhouse as the desire was to conduct experimental tests under controlled conditions.

MATERIALS AND METHODS

Inoculum production

MV spores [*M. verrucaria* (IMI 361690)] were cultured in petri dishes on potato dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI, USA) incubated at 25°C for 5 days. Conidia (spores) were harvested by rinsing plates with sterile H_2O and conidial concentrations were determined using a hemacytometer. A fermenter was inoculated with MV spores and allowed to grow on a proprietary liquid medium for 48-72 hr. The MV mycelia product produced via fermentation was harvested and stored at 4°C until use. Concentrations of the mycelia formulations used in these tests were based on fresh weight and are presented as mg fresh weight mL^{-1}.

Chemicals

Quinclorac was a technical grade (98% purity) product obtained from Chem Service (West Chester, PA, USA). All other chemicals used in these experiments were of reagent grade quality or higher.

Bioassays, greening tests and chlorophyll analysis

Seeds of hemp sesbania and sicklepod (harvested from a weed nursery, USDA-ARS, Stoneville, MS, USA) were mechanically scarified and germinated in rolled paper towel cylinders, and grown hydroponically under continuous darkness for 4 days as described previously (Hoagland 1995). Five excised shoot segments (upper 10 mm) containing cotyledons were cut using a razor blade, and then placed in Petri dishes containing water (control), MV, quinclorac, or MV plus quinclorac at various concentrations. All treatments solutions (control, quinclorac, MV, and MV plus quinclorac) contained a non-ionic surfactant, Silwet L-77 at 0.1%, v/v (Lovelace Industries, Greeley, CO, USA). The dishes containing etiolated seedling tissues were then placed in an environmental chamber (Lab-Line, Biotronette Mark III, Melrose Park, IL, USA) under continuous light [100 $\mu E\ m^{-2}\ s^{-1}$, photosynthetic photon flux (PPF)] as determined with a light meter (LI-COR, Inc., Lincoln, NE, USA) at 27°C. After 72 h exposure to light and the treatment regimes, elongation, fresh weight and chlorophyll accumulation were measured or analyzed. Chlorophyll was extracted and quantitatively determined spectrophotometrically using dimethyl sulfoxide (Hiscox and Israelstam 1979; Barnes *et al.* 1992).

Greenhouse tests

Kudzu seeds (Adams-Briscoe Seed Co., Jackson, GA, USA) were planted and grown in a potting soil mixture in a greenhouse. Greenhouse temperatures were ranged at 28-30°C (day) and 22-25°C (night) at a 16 photoperiod. Light intensity averaged 1650 $\mu Em^{-2}\ s^{-1}$ (PPF), measured with a light meter at midday. Dilute fertilizer solution (N, P, K) provided nutrients. When the plants were 2-weeks-old, they were utilized for the experimental treatments. All treatments solutions (control, quinclorac, MV, and MV plus quinclorac) contained a non-ionic surfactant, Silwet L-77 as described above, and were applied using hand-held sprayers. Visual assessment of injury and fresh and dry weights were measured 7 days after treatment (DAT).

Quantification of potential interactions of MV and quinclorac

Potential interactions between the bioherbicide (MV) and the herbicide (quinclorac) in mixtures were analyzed according to Colby (1967), using the formula, $E = X_A Y_B/100$, in which X_A and Y_B represent injury (reduction of chlorophyll content or dry weight reduction) as a percentage of the control, when bioherbicide A (MV) was used at dosage p and herbicide B (quinclorac) used at dosage q, respectively. E is the expected survival as a percentage of the control for mixture of A and B at dosages p and q. The observed response is obtained experimentally by comparing the activity of the single agents with the activity of mixtures containing the same concentration of the agents as applied singly. A deviation from the expected response, as calculated from the level of interaction R between the expected and the observed response of the two agents, would indicate synergism or antagonism. By definition, additive interactions are present if $R = 1$, synergism occurs $R > 1$, and antagonism occurs if $R < 1$. Due to the inherent biological variability of the test systems, synergism is considered significant if $R = 1.5$ and antagonism is considered significant if $R = 0.5$. Additive interactions are noted when R is between 0.5 and 1.5 (Gisi *et al*. 1985).

Statistical analyses

Experimental units in the laboratory bioassays consisted of 5 excised plants per treatment, the treatments were replicated three times. In greenhouse tests, each treatment unit for kudzu contained 4 plants, with each treatment set up in triplicate. All experiments in the laboratory and greenhouse were repeated in time. All experiments were arranged in randomized complete block designs and means were separated using Fisher's Least Significant Difference at $P = 0.05$. Error bars in figures represent ± one standard error of means.

RESULTS AND DISCUSSION

Effects of quinclorac alone on sicklepod and hemp sesbania

High purity quinclorac alone at low concentrations caused swelling in sicklepod (**Fig. 1A**) and hemp sesbania (**Fig. 2A**), but the effect was subjugated in both species at higher herbicide concentrations of about 0.19 mM and higher. These effects are visually apparent and are substantiated in the growth measured as fresh weight accumulation (**Figs. 1B, 2B**). Growth was inhibited in both species as concentra-

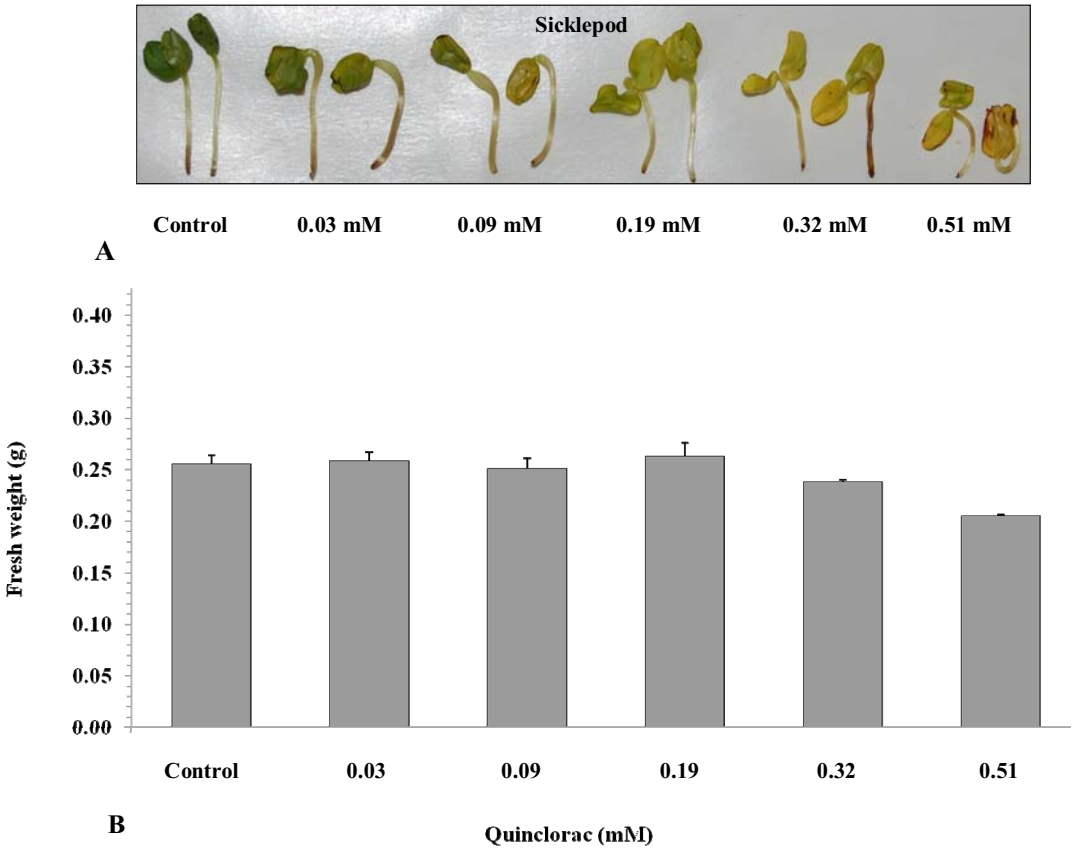

Fig. 1 Effects of quinclorac at several concentrations on growth and development of excised, etiolated sicklepod seedling segments, 72 h after treatment and exposure to light. (**A**) Visual depiction of injury; (**B**) injury expressed as fresh weight reduction. Error bars represent ± one standard error of means. Regression followed a linear model: $y = 0.275 – 0.01$; $r^2 = 0.557$.

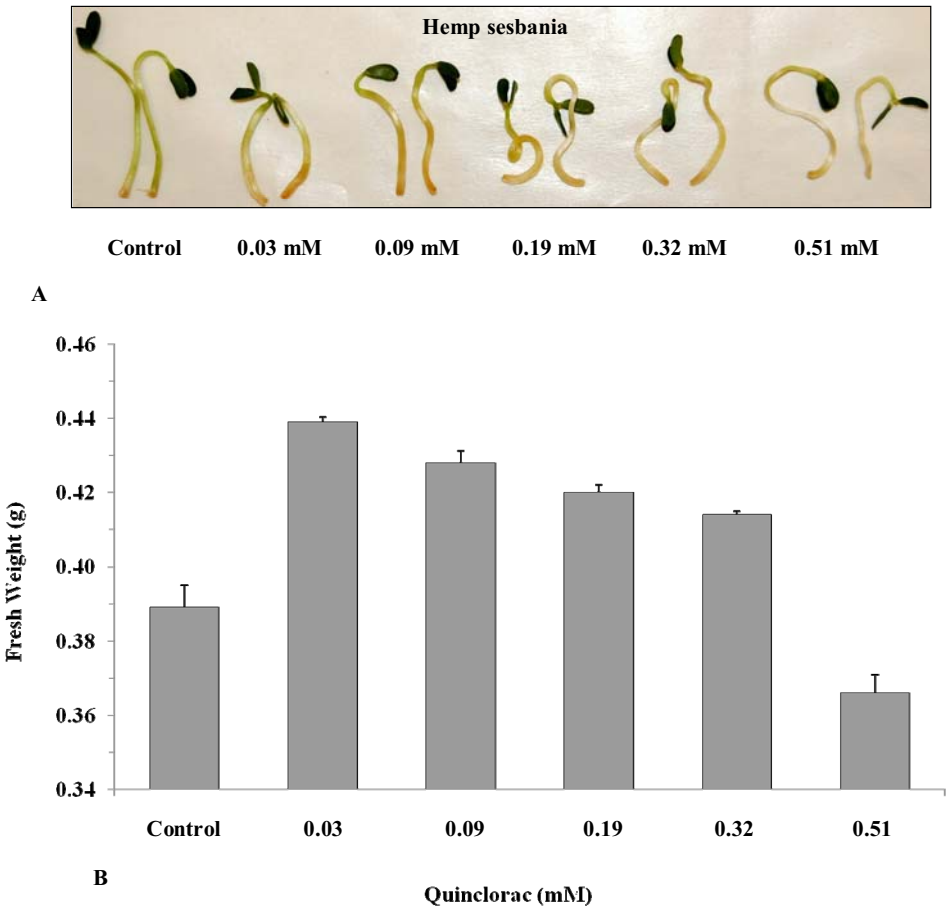

Fig. 2 Effects of quinclorac at several concentrations on growth and development of excised, etiolated hemp sesbania seedling segments, 72 h after treatment and exposure to light. (**A**) Visual depiction of injury; (**B**) injury expressed as fresh weight reduction. Error bars represent ± one standard error of means. Regression followed a linear model: $y = 0.428 – 0.005$; $r^2 = 0.631$.

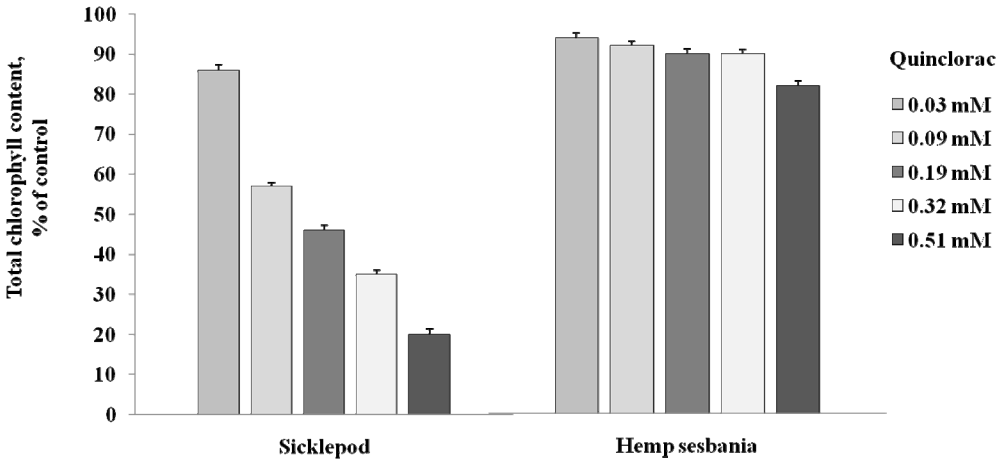

Fig. 3 Effects of quinclorac concentration on chlorophyll accumulation in etiolated, excised tissues of sicklepod and hemp sesbania, 72 h after treatment and exposure to light. Error bars represent ± one standard error of means. Regression followed a linear model: $y = 95 – 15.4$; $r^2 = 0.96$.

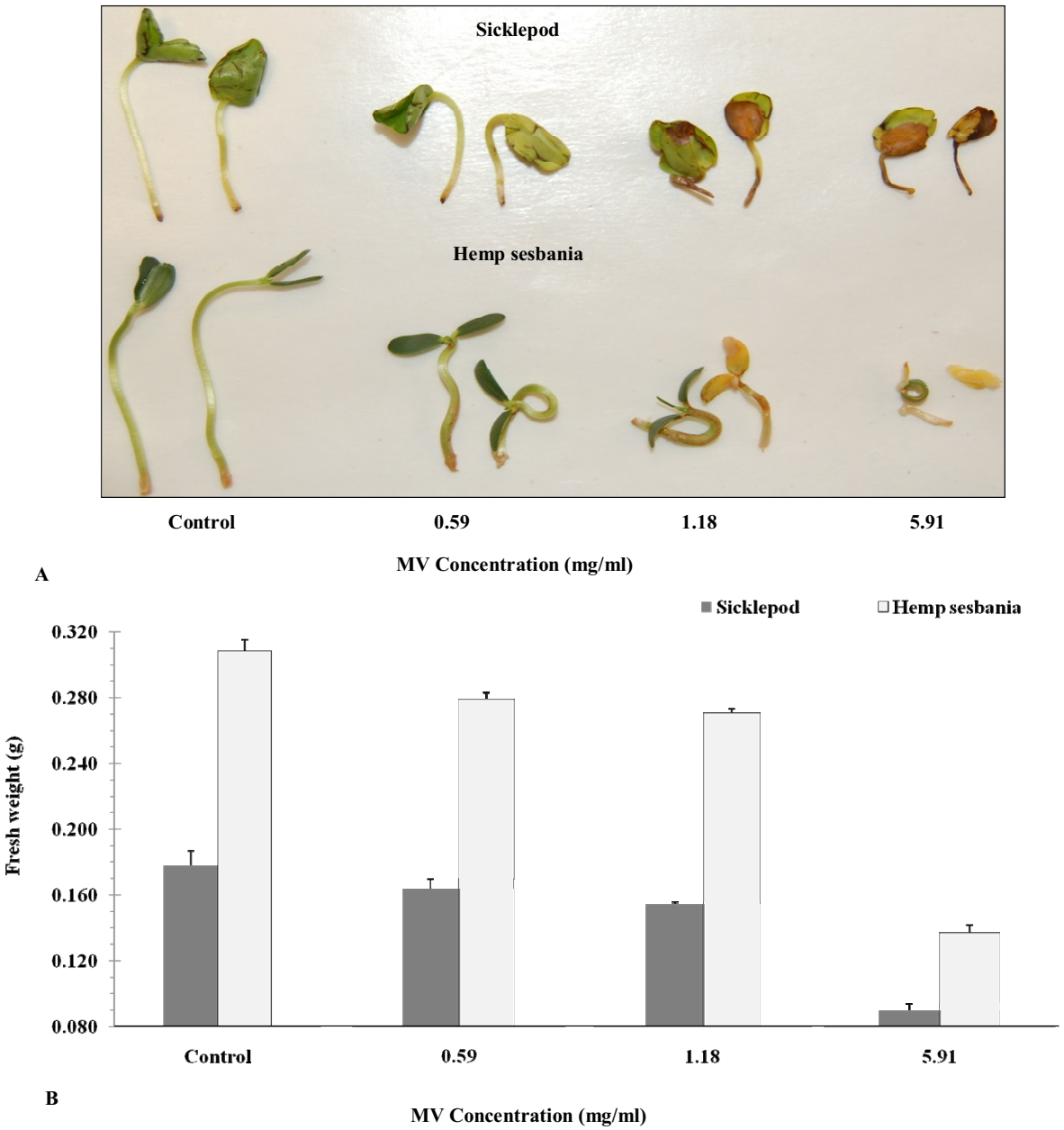

Fig. 4 Effects of *Myrothecium verrucaria* (MV) on growth and development of excised, etiolated sicklepod and hemp sesbania seedling segments, 72 h after treatment and exposure to light. (**A**) Visual effects of MV at several concentrations on these excised tissue segments; (**B**) fresh weight data representing MV effects. Error bars represent ± one standard error of means. Regression followed a linear model: $y = 0.379 – 0.052$; $r^2 = 0.784$.

tion increased. Quinclorac also caused some increased twisting of curving (typical of an auxin-type herbicide) of hemp sesbania stems at several concentrations, but this effect was not pronounced in sicklepod tissue segments. As the concentration of quinclorac increased, chlorophyll accumulation in the greening plant segments decreased in both species, but the effect was much more pronounced in sicklepod (**Fig. 3**). In sicklepod the effect was direct and linear. Visually, chlorophyll was substantially reduced in the cotyledons of sicklepod, whereas in hemp sesbania cotyledons, chlorophyll was reduced only slightly, but decreased dramatically in the stem tissue of hemp sesbania as the quinclorac concentration increased (data not shown). Since herbicides, including quinclorac are differentially absorbed and translocated in different plant species, this may also be occurring in sicklepod and hemp sesbania. The differential effect could also be due to different rates of metabolism of the herbicide.

Effects of MV alone on sicklepod and hemp sesbania

MV treatment alone significantly inhibited growth and chlorophyll accumulation in sicklepod and hemp sesbania, but only slight or no swelling was exhibited (**Figs. 4A**). Reductions in growth (visual and measured) were generally proportional to the mycelia concentration (**Figs. 4A, 4B**). Chlorophyll accumulation was directly proportional to increased MV mycelia concentration of this bioherbical fungus in both species (**Fig. 5**). The effects on chlorophyll in these tissues caused by MV differed from that caused by quinclorac which showed that reduction of chlorophyll accumulation was more prominent in sicklepod and that hemp sesbania was relatively insensitive with regard to this parameter. Although we have examined the effects of MV on several invasive weeds (Boyette *et al.* 2007; Hoagland *et al.* 2007; Boyette *et al.* 2008a) the levels of MV utilized were much greater than the very low amounts used in these bioassays.

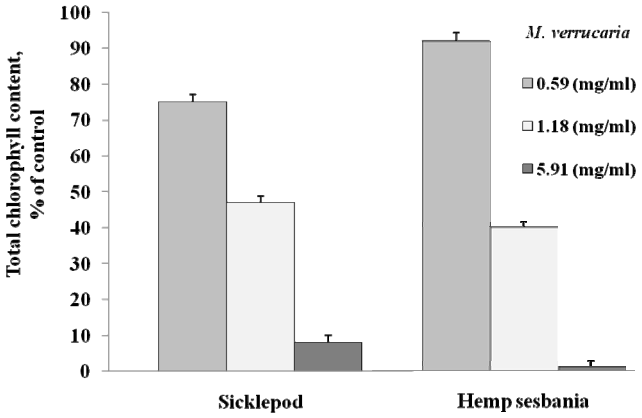

Fig. 5 Effects of *Myrothecium verrucaria* (MV) on chlorophyll accumulation in excised, etiolated sicklepod and hemp sesbania seedling segments, 72 h after treatment and exposure to light. Error bars represent ± one standard error of means.

Effects of combinations of MV and quinclorac at several concentrations

Low concentrations of MV (Boyette *et al*. 2008c) and quinclorac (preliminary tests) were chosen to provide a range of effects on growth and chlorophyll content rather than rapid mortality that would be caused by high concentrations. Low concentrations of MV plus quinclorac induced swelling in sicklepod, but not hemp sesbania (**Fig. 6**). This swelling phenomenon dissipated in sicklepod as the MV and/or quinclorac concentrations increased. Furthermore, as the MV mycelia concentration increased, there was greater tissue maceration in both sicklepod and hemp sesbania. However, hemp sesbania apperared somewhat more susceptible to tissue maceration/degradation caused by the action of these components. Although quinclorac alone caused no visible maceration of these tissues, the combined treatment of MV and quinclorac increased this factor in both species, and at both concentrations tested. Analysis of the chloro-

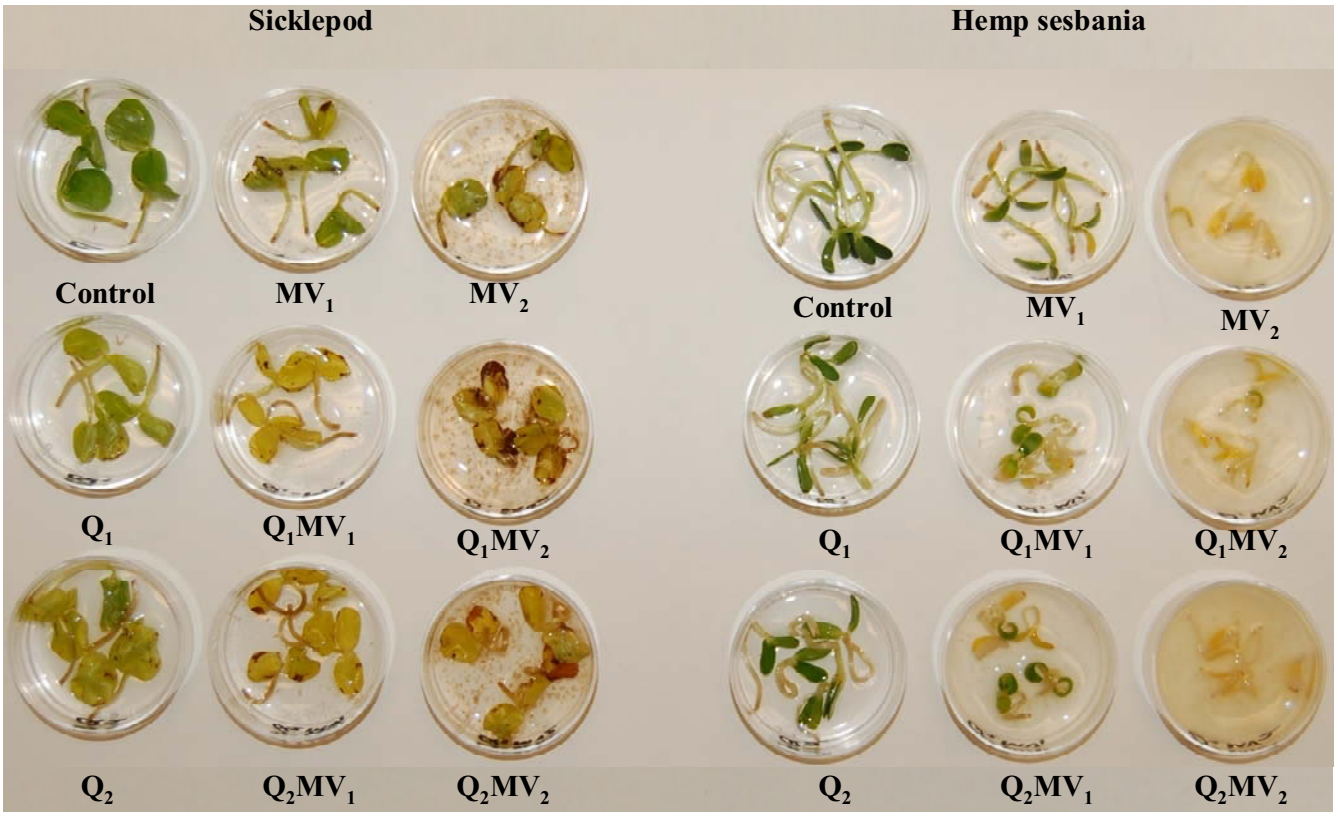

Fig. 6 Effects of *Myrothecium verrucaria* (MV) and quinclorac alone and in their combination on excised, etiolated sicklepod and hemp sesbania seedling segments, 72 h after treatment and exposure to light. Control = H_2O; MV_1 = 0.89 mg/mL; MV_2 = 3.6 mg/mL; Q_1 = 0.09 mM; Q_2 = 0.51 mM.

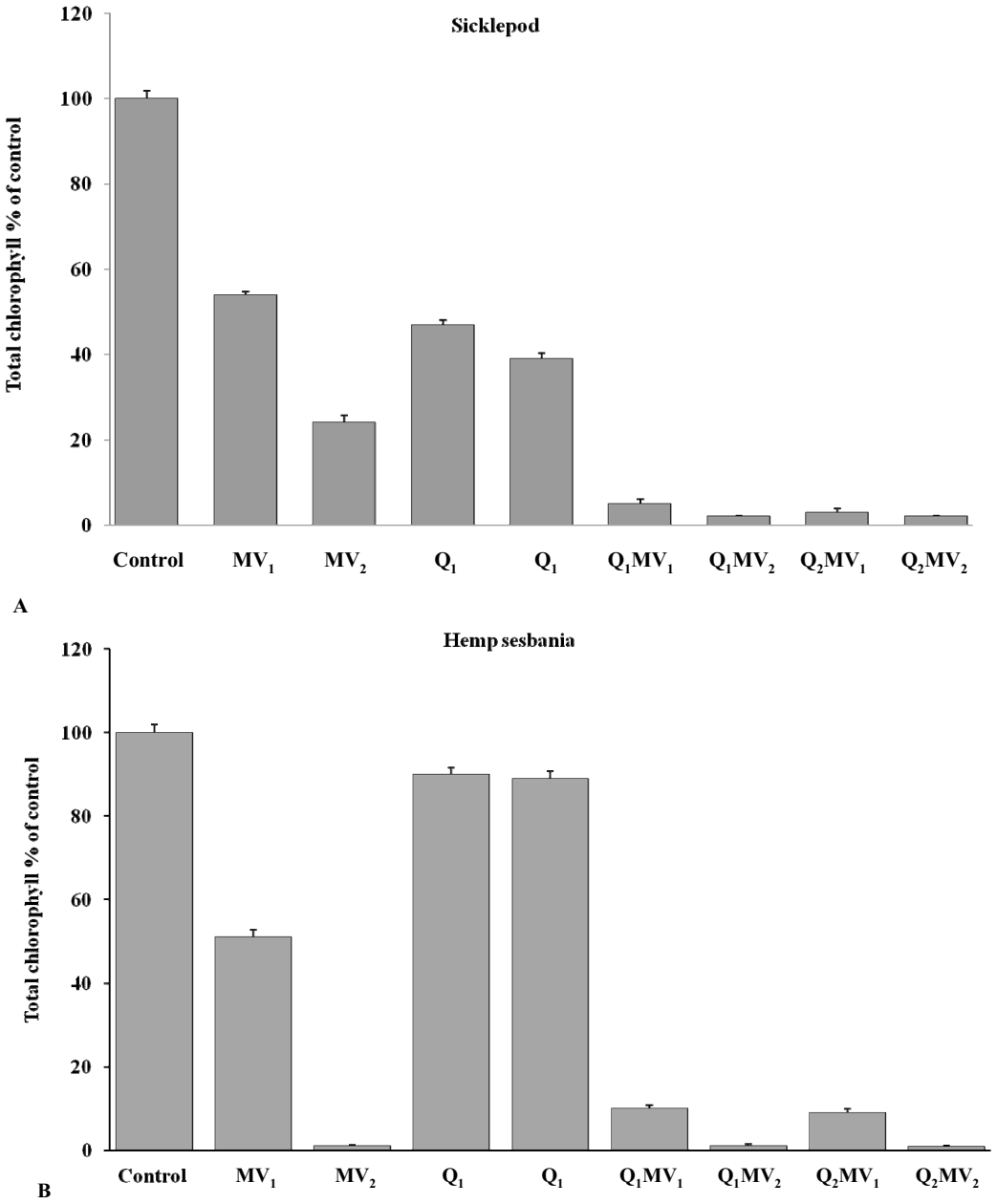

Fig. 7 Effects of *Myrothecium verrucaria* (MV) and quinclorac (Q) alone, and their combination on chlorophyll accumulation in excised, etiolated sicklepod (A), and hemp sesbania (B) seedling segments, 72 h after treatment and exposure to light. Treatments: Control = H_2O; MV_1 = 0.89 mg/mL; MV_2 = 3.6 mg/mL; Q_1 = 0.09 mM; Q_2 = 0.51 mM. Error bars represent ± one standard error of means.

phyll content after treatment with MV and quinclorac alone and in combinations showed dramatic reductions of this pigment in hemp sesbania and sicklepod segments (**Fig. 7A, 7B**). These results indicate that interactions of this herbicide/bioherbicide combination do occur, suggesting important additive and/or synergistic effects. Analysis of this data (Colby 1967; Gisi *et al*. 1985) for possible synergistic, antagonistic, or additive interactions demonstrated that several concentrations of MV and quinclorac applied as a mixture produced synergistic effects on chlorophyll reduction (**Table 1**). Previously we have shown additive and synergistic effects on MV and glyphosate applied to other invasive weeds (Boyette *et al.* 2006, 2008a), but generally those results were achieved at higher concentrations and under field conditions.

Effects of MV, quinclorac, and their combination on kudzu plants

Visually, interactions of this herbicide and bioherbicide are apparent in kudzu plants (**Fig. 8A**) and in a closer view in excised leaves of treated kudzu plants (**Fig. 8B**) Combinations of MV plus quinclorac prevented severe swelling in kudzu (caused by quinclorac alone), and exhibited additive and/or synergistic injury and/or mortality effects. Growth analysis (fresh weight and dry weight determinations) (**Fig. 9**) and the visual effects (**Figs. 8A, 8B**) suggest an additive effect on kudzu when MV and quinclorac application is made simultaneously. Results of the interaction analysis (Colby 1967; Gisi *et al*. 1985) of the kudzu data on a dry weight basis supports the concept that the effects of combining MV and quinclorac at these concentrations under the conditions outlined here resulted in an additive effect (**Table 2**). Results in the greenhouse indicate that very low levels of fungus and herbicide can cause high weed control efficacy when these two components are applied simultaneously.

These bioassays and greenhouse tests show the herbicide quinclorac may act additively and/or synergistically with MV on sicklepod, hemp sesbania and kudzu depending on the parameter measured. The exact reason for this interaction is not understood. In another study, quinclorac was highly inhibitory to growth of corn tissue and significantly decreased total chlorophyll content in the light, but not

Table 1 Interaction between *Myrothecium verrucaria* and quinclorac relative to reduction of total chlorophyll content (expressed as percent of control) in excised hemp sesbania and sicklepod tissues as analyzed using Colby's method (Colby 1967; Gisi *et al*. 1985).

Treatment	Observed[a]		Expected[b]		R[c]	
	Chlorophyll content					
	HS	SP	HS	SP	HS	SP
MV_1	54	51	54	51	1	1
MV_2	24	2	24	2	1	1
Q_1	47	90	47	90	1	1
Q_2	39	89	39	89	1	1
$MV_1 + Q_1$	5	10	25	46	5.0 *	4.6 *
$MV_1 + Q_2$	3	9	21	45	7.0 *	5.0 *
$MV_2 + Q_1$	2	2	11	2	5.5 *	1
$MV_2 + Q_2$	2	1.5	9	1.8	4.5 *	1

[a] Observed chlorophyll content (percent of control) extracted from plant tissue segments.
[b] Expected values were determined using the Colby (1963) equation, $E = X_A Y_B/100$, where E is the expected chlorophyll content (expressed as percent of control) with components A+B at concentrations p and q for MV and quinclorac, respectively; X_A is the observed chlorophyll content with A (MV), and Y_B is the observed chlorophyll content with B (Q, quinclorac); [c] R the ratio between the expected and observed survival (R = expected/observed); R values ≥ 1.5 are considered to be synergistic (Gisi *et al*. 1985) and additive interaction are noted when R is between 0.5 and 1.5.

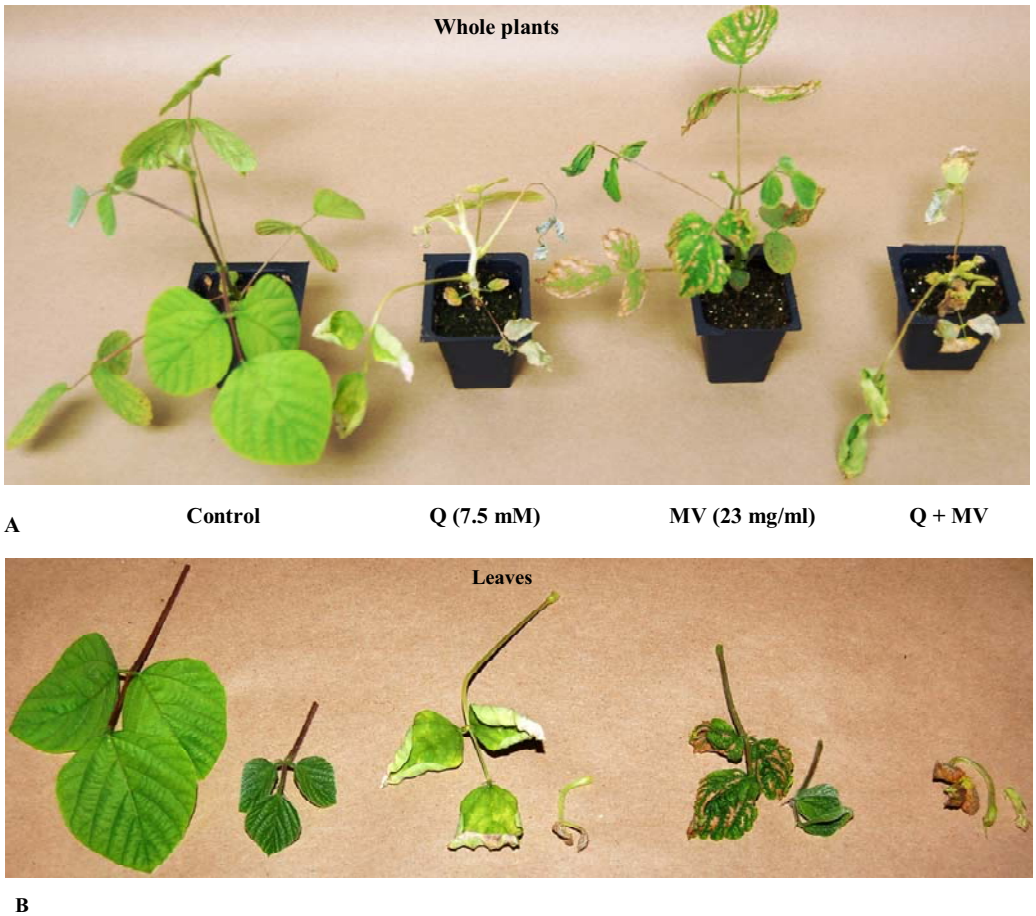

Fig. 8 Effects of *Myrothecium verrucaria* (MV) at 23 mg fresh weight mL^{-1}, quinclorac (Q) (7.5 mM), and their combination at these concentrations on kudzu plants 7 days after treatment under greenhouse conditions. (**A**) Depicts injury symptoms on whole plants; (**B**) depicts close view of injury of leaves from whole plants treated at same concentrations.

under dark conditions (Sunohara and Matsumoto 1997). Quinclorac also increased ethylene production under light conditions, (but not in the dark), and the herbicide did not bind with a membrane-bound auxin receptor in corn. Further, these authors found that quinclorac enhanced ethylene biosynthesis and herbicide-induced chlorosis in corn leaves were light dependent. Continuous light exposure was used in the bioassays of MV and quinclorac, but a project to examine the effects of their combination in light versus dark treatments might reveal important information related to the mechanism of each component.

In another study at the ultra-structural level, MV alone caused a rapid (within 1 h after treatment) detachment of the protoplast from the cell wall accompanied by the appearance of broken off plasmodesmata that remained in the wall of kudzu tissues (Hoagland *et al*. 2008). These ultra-structural symptoms occurred well in advance of the appearance of any fungal growth structures. Ultra-structurally MV causes protoplast detachment from the cell wall in kudzu, accompanied by broken-off plasmodesmata retained in cell walls. How these ultra-structural effects correlate with swelling, or the prevention of swelling in some of these tissues is not presently known. One possible explanation relates to the fact that auxenic herbicides such as quinclorac cause cell wall-loosening as part of their herbicidal effect, and the observation that MV causes a detachment of the protoplast from the cell wall, thus eliminating the ability of the cell wall to form new polysaccharides. These two effects on wall integrity appear to act in a synergistic manner, resulting in a loosened and weakened cell wall,

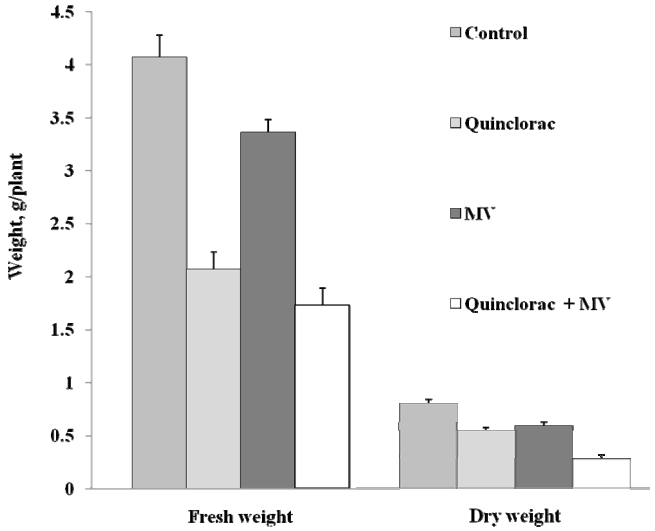

Fig. 9 Effects of *Myrothecium verrucaria* (MV) at 23 mg mL^{-1}, quinclorac (7.5 mM), and their combination at these concentrations on fresh weight and dry weight accumulation of 2-week-old kudzu seedlings, 7 days after treatment. Error bars represent ± one standard error of means.

Table 2 Interaction between *Myrothecium verrucaria* and quinclorac on fresh and dry weight reduction (expressed as percent of control) in kudzu plants tested under greenhouse conditions.

Treatment	Observed[a]		Expected[b]		R[c]	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	100	100	100	100	1.0	1.0
Q	50	67	50	67	1.0	1.0
MV	83	68	83	68	1.0	1.0
Q+MV	41	36	42	46	1.0	1.3

[a] Observed chlorophyll content (percent of control) extracted from kudzu tissue.
[b] Expected values were determined using the Colby (1963) equation, $E = X_A Y_B/100$, where E is the expected chlorophyll content (expressed as percent of control) with components A+B at concentrations p and q for MV and quinclorac, respectively; X_A is the observed chlorophyll content with A (MV), and Y_B is the observed chlorophyll content with B (Q, quinclorac); [c] R the ratio between the expected and observed survival (R = expected/observed); R values ≥ 1.5 are considered to be synergistic (Gisi *et al*. 1985) and additive interaction are noted when R is between 0.5 and 1.5.

leading to enhanced phytotoxicity of the combination of the biocontrol agent and herbicide. Further ultra-structural analysis of MV and quinclorac interactions in these tissues is warranted.

Further characterization of these findings at the molecular level is also being pursued and investigated in the laboratory and extended with greenhouse and field testing. Ethylene biosynthesis may be closely related to the action of quinclorac in monocots, but whether it plays any role in dicots such as sicklepod, hemp sesbania or kudzu remains obscure. Studies of interactions are complex, especially when the proposed mode of action of the herbicide in question is not well understood, and the mode of action of the bioherbicide used here is completely unknown. However, further information will be important to characterize such roles of herbicide interactions in infectivity, necrosis and death of target weeds. To aid in the development and improvement of a commercial product of MV, knowledge of its virulence factors and its interactions with other agochemicals is crucial.

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