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Table: -, Figs : 5

Effects of *Myrothecium verrucaria* on morning-glory (*Ipomoea*) species

R.E. HOAGLAND[1], T.S. MCCALLISTER[1], C.D. BOYETTE[2], M.A. WEAVER[2] and R.V. BEECHAM[3]

[1]USDA-ARS, Crop Production Systems Research Unit, Stoneville, MS 38776
E. Mail: Bob.Hoagland@ARS.USDA.GOV

ABSTRACT

During field testing of a bioherbicidal strain of the fungus *Myrothecium verrucaria* (MV) for control of spurges and purslanes in tomato test plots in summer of 2005, we noted extensive damage to volunteer morning-glory (*Ipomoea* spp.) seedlings. This observation prompted investigations on the biological control efficacy of MV on various *Ipomoea* species under a controlled environment. Seven morning-glory species [ivyleaf (*Ipomoea hederacea*), moonvine (*Ipomoea turbinate*), palmleaf (*Ipomoea wrightii*), pitted (*Ipomoea lacunosa*), multi-color (*Ipomoea tricolor*), moonflower (*Ipomoea alba*), and cypressvine (*Ipomoea quamoclit*)] were grown in greenhouse and tested at first to second leaf growth stage. MV spores (10^7spores mL^{-1}) were formulated in Silwet L-77 surfactant (0.2 %, v/v) or an invert emulsion containing this surfactant. Plants were treated either with Silwet (0.2%, v/v) alone (control), invert emulsion plus Silwet, MV plus Silwet, or MV plus Silwet plus invert emulsion via spray application. After application, the plants were placed in a dew chamber (15-18 h) and then transferred to a greenhouse. Plant injury and disease progression were assessed visually and fresh and dry weights were determined at the end of tests (7 days after treatment). Some of these species exhibited more tolerance than others to spray applications of MV plus Silwet, depending on the time after treatment. Compared to MV alone treatments, formulations of MV plus the invert emulsion promoted injury symptomology in pitted and moonvine morning-glories, but caused less disease symptomology than MV alone in palmleaf seedlings. There were no significant differences in disease symptomology of the MV alone and MV plus invert treatments in the other species. Overall, the results indicate some differential injury effects of MV on closely related species, i.e., *Ipomoea* (morning-glories), and that the invert emulsion can increase the efficacy of MV in certain instances.

Keywords: Biocontrol agent, bioherbicide, morning-glory, *Myrothecium verrucaria*, weeds

INTRODUCTION

Annual morning-glories (*Ipomoea* spp.) are herbaceous, viney, climbing and creeping plants (16). Although cultivars of some morning-glory species are ornamental but as a group they are considered most troublesome weeds in many crops throughout U. S. (33). The morphology and aggressiveness of morning-glories vary from species to species, but generally each of these species can cause problems to farmers and gardeners throughout the U.S. Pitted morning-glory (*Ipomoea lacunosa*) has been cited as the most common, and the second most troublesome weed in Mississippi soybean production (34). Recently, differences found in pitted morning-glory collections from eleven states,

Correspondence Author; [2]USDA-ARS, Biological Control of Pests Research Unit, Stoneville, MS 38776; [3]Mississippi Valley State University, Itta Bena, MS 38941

indicated that this species exists as several morphological ecotypes (27). Morning-glories can reduce soybean yield up to 75%, and full-season interference of annual morning-glories can increase lodging and reduce mechanical harvest efficiency (3). The spread of these onerous weeds has grown at an alarming rate and there is urgent need to find new control measures.

The use of biological control agents, such as bacterial and fungal phyto-pathogens, to manage weeds is currently of interest to researchers and the agriculture industry (13,18,30). Numerous pathogens have been discovered that have bioherbicidal potential to control a large spectrum of weeds. One current example is *Myrothecium verrucaria* (strain number: IMI Accession No. 361690, here-to-for referred to as MV) originally isolated from the weed sicklepod (*Senna obtusifolia* L.) (28). This organism controls several weeds, when used as bioherbicide (2,8,9,19,20,28). Although this strain has potent bioherbicidal properties, it also produces undesirable trichothecenes (mycotoxins) (1, 5). *Myrothecium* species are also of interest because they commonly occur as soil inhabitants of temperate and tropical regions (15) and some species or strains produce wide diversity of metabolites (enzymes, antibiotics, mycotoxins, etc.) (14,17,24). Although some of these species and their metabolites possess allelopathic properties, generally the precise roles and/or mechanisms of action of specific metabolites as allelopathic agents are unclear at this time. *Myrothecium* species have been shown to be causal agents of disease in legume crops (22) and nematodes (29).

An early report indicated that MV (IMI No. 361690) had no effect on dry weight reduction or mortality of pitted morning-glory (28). Other reports on this MV strain (361690) indicated only 30% or less reduction in dry weight of certain morning-glories treated with MV (2). Neither of these studies showed that MV was a very effective bioherbicide against annual morning-glories.

Conidia of another *M. verrucaria* strain isolated from the leafy spurge (*Euphorbia esula* L.) exhibited some virulence to several morning-glory species [tall morning-glory (*I. aquatica* Forsk), ivyleaf morning-glory (*I. hederacea*), and red morning-glory (*I. coccinea L.*) (23)]. All of these reported results, coupled with recent observations during field tests, i.e., that our MV strain injured young seedlings of some volunteer morning-glory species, prompted a more in-depth study of MV effects on several diverse species of morning-glories. Therefore our objectives were to determine the differential effects of MV (No. 361690) on several species of annual morning-glories and to ascertain if an invert emulsion formulation of the fungus could enhance its efficacy under controlled (greenhouse) conditions.

MATERIALS AND METHODS

MV Source and Culture: MV isolate, (IMI 361690) was used throughout the studies presented here. Inocula (conidia) of MV for all experiments were produced in Petri dishes containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA)[1]. The dishes were inoculated by flooding each dish with 3 mL suspension containing 2 x 10^6 conidia mL^{-1}. The inoculated dishes were inverted and placed on open-mesh wire shelves

[1] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

in an incubator (Precision Scientific Inc., Chicago, IL, USA) at 28°C for 5 days. Photoperiods (12-h) were provided by two 20-W, cool-white fluorescent lamps positioned in the incubator door. Light intensity at dish level was approximately 200 µmol m^{-2} s^{-1} (photo-synthetically active radiation) as measured with a light meter. After 5 days, conidia were rinsed from the confluent lawns in each Petri dish with sterile, distilled water. The numbers of conidia were estimated with hemacytometers (Thermo Fisher Scientific, Waltham, MA, USA) and adjusted to the desired concentration (1 x 10^7 conidia mL^{-1}) with sterile distilled water.

Seed and Chemical Sources: Morning-glory (*Ipomoea* spp.) seeds used in these tests were purchased from Herbiseed Co. (United Kingdom; www.heribseed.com). Tested species were: *I. alba* [moonflower (CLYAC)2], *I. hederacea* [ivyleaf (IPOHE)], *I. lacunosa* [pitted (IPOLA)], *I. tricolor* [multicolor (IPOTO)], *I. wrightii* [palmleaf (IPOWR)], *I. turbinate* Lag. [moonvine (CLYMU)], and *I. quamoclit* [cypressvine (IPOQU)]. All seeds were mechanically scarified using a rotary canister lined with sandpaper (Forsberg's. Inc., Thief River Falls, MN 56701, USA) to permit imbibition of water through their seed coats that is required for germination. The invert emulsion consisted of combinations of high purity paraffinic oil, lanolin and water and was prepared as described elsewhere (25). Briefly, the oil component of invert emulsion consisted paraffinic oil (Orchex 797; Exxon Corp., Baytown, TX, USA) (777.5 g l^{-1}), a monoglyceride emulsifier (Myverol 18-99; Eastman Chem. Prod., Inc., Kingsport, TN, USA) (14.5 g l^{-1}), paraffin wax (Strohmeyer & Arpe Co., Inc., Short Hills, NJ, USA) (74.25 g l^{-1}), and lanolin (93g l^{-1}). A stable invert emulsion was formed when equal parts of the oil component and water were combined and stirred briskly by hand with a stirring rod for 2-3 min.

Plant Propagation: About 100 scarified seeds of each *Ipomoea* species were hand-sown into individual trays of 50:50 mixture of 1:1 commercial potting mix/soil, containing fertilizer [N:P:K (13:13:13)]. Trays were placed inside a greenhouse (20-24 °C, 16 h photoperiod supplied with supplemental lighting). Trays were watered with de-ionized water. Seedlings emerged in 2 to 4 days after planting (DAP). At the cotyledon growth stage (ca. 6 to 7 DAP), seedlings of uniform size were transplanted into pots (9 x 7 cm) containing the potting soil mixture described above. Seedlings were watered with de-ionized water and maintained in the greenhouse until treatment.

Application of *Myrothecuim verrucaria* Spore Formulations: Five seedlings from each species at 1 to 2 leaf growth stage were sprayed using fully-charged, hand-held compressed air spray canisters (Crown Spra-Tool, North American Professional Products, Woodstock, IL, USA) to run-off (ca. 300 L ha^{-1}), with each treatment [Silwet (control), MV plus Silwet, invert emulsion plus Silwet, and MV plus invert emulsion plus Silwet]. All treatments contained 0.2% (v/v) Silwet L-77 surfactant. Applications were administered in a bio-safety cabinet (NuAire, Model No. NU-425-400, Plymouth, MN, USA). After treatment, the seedlings were placed in a dew chamber (Percival Scientific,

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[1] Bayer Codes for weed species (4).

Model No. 1-35 DL, Boone, IA, USA) at 25 °C for 16 to 18 h. After the dew treatment, the plants were placed in a greenhouse for further growth, observation, and measurements.
Determination of MV Effects on Plant Growth: Seedlings were visually examined and photographed for injury symptoms at 20 to 22 h after treatment (HAT) and at 3 days after treatment (DAT). Plant shoot heights (lengths measured to the nearest mm), and shoot fresh and dry weights (measured to the nearest one-hundredth of a g) were determined 7 DAT after excising the shoots at soil level. Excised shoots used for dry weight determinations were placed in paper bags, labeled, and dried in a forced-air oven at 90 °C for 72 h prior to weighing.

Experimental Design and Statistical Treatments: A randomized complete block experimental design was used. Each treatment consisted of 4 to 6 plants and all treatments were set-up in triplicate. All experiments were repeated in time. The data were statistically compared using analysis of variance (ANOVA) at the 5% probability level. Values presented are the means of replicated experiments. When significant differences were detected by the *F*-test, means were separated with Fisher's protected LSD test at the 0.05 level of probability. Data were combined for analysis (ANOVA) and presentation, and the data in Figures 5 and 6 have been normalized (percent of control), and presented as mean values (represented as histogram bars) on a per plant basis for each treatment and measured parameter. Disease progression or severity of MV on these species was monitored at several intervals over a 7- day period using a modified visual disease severity rating scale (21), where: 0 represented no infection, and 0.2, 0.4, 0.6, 0.8 represented 20, 40, 60, and 80 % leaf and stem lesion coverage/injury, respectively, and 1.0 = plant mortality. Data were analyzed using standard mean errors and best-fit regression analysis. Error bars are ±1 SEM.

RESULTS AND DISCUSSION

Visual Effects of Treatments: Comparison of overall growth and visual effects (3 DAT) of these morning-glory species treated with Silwet-L 77 (the control), invert emulsion plus Silwet, MV spores plus Silwet and MV plus Silwet plus invert emulsion demonstrated no significant growth or injury effects caused by Silwet alone at this concentration, but that the invert emulsion plus Silwet caused slight effects in some species (Figure 1). However, it was clear that growth reductions and/or mortality occurred in some species treated with MV plus Silwet and in the MV plus Silwet plus invert emulsion treatments 3 DAT (Figure 1). At 4 DAT, pitted morning-glory seedlings exhibited injury and mortality symptoms more quickly than moonvine, and pitted also showed some susceptibility to treatment with the invert emulsion plus Silwet formulation at this time point (data not shown).

At 7 DAT, both the control plants (Silwet treated) and the invert emulsion plus Silwet treated plants had grown significantly, and plant growth inhibition and mortality were more evident in many of the other species that were treated with formulations containing MV. For example, at 7 DAT both pitted and moonvine seedlings treated with Silwet alone or the invert emulsion plus Silwet were much more robust than the plants treated with the MV formulations (Figure 2). Pitted morning-glory seedlings were more

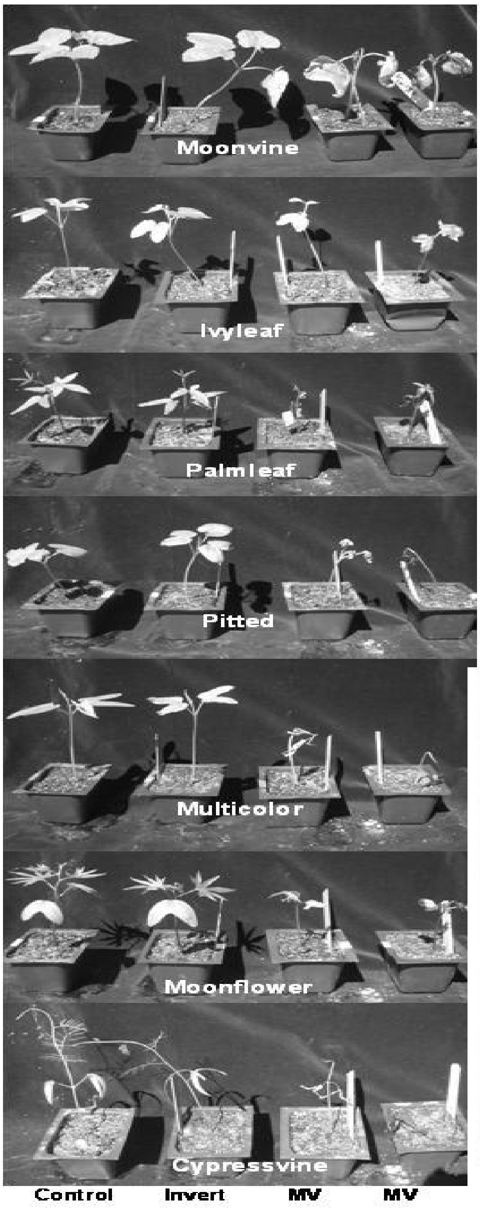

Figure 1. Visual effects of 0.2% Silwet alone (control), invert emulsion plus Silwet, and MV combined with either Silwet or invert emulsion plus Silwet on seven species of morning-glory seedlings, 3 DAT.

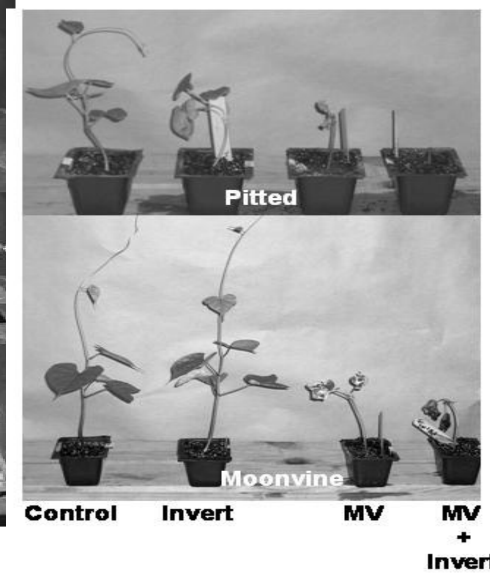

Figure 2. Effects of pitted and moonvine morning-glory seedlings, 7 DAT with Silwet, invert emulsion plus Silwet, and MV combined with either Silwet or with the invert emulsion plus Silwet.

susceptible to the invert emulsion plus Silwet treatment and to MV formulated with either Silwet, or with invert emulsion plus Silwet, compared to moonvine seedlings (Figure 2).

Analysis of MV disease progression over a 7- day period indicated that disease severity was greatest in IPOTO, IPOWR, IPOLA and IPOQU, with lesser severity occurring in CLYAC and CLYMU, and the least affected was IPOHE (Figure 3). There were no significant differences in the disease progression of four species that were most sensitive to MV. In species with intermediate ratings, disease in CLYMU progressed much slower than CLYAC in the early stages after application, but at 7 DAT both species exhibited a disease rating of 0.85.

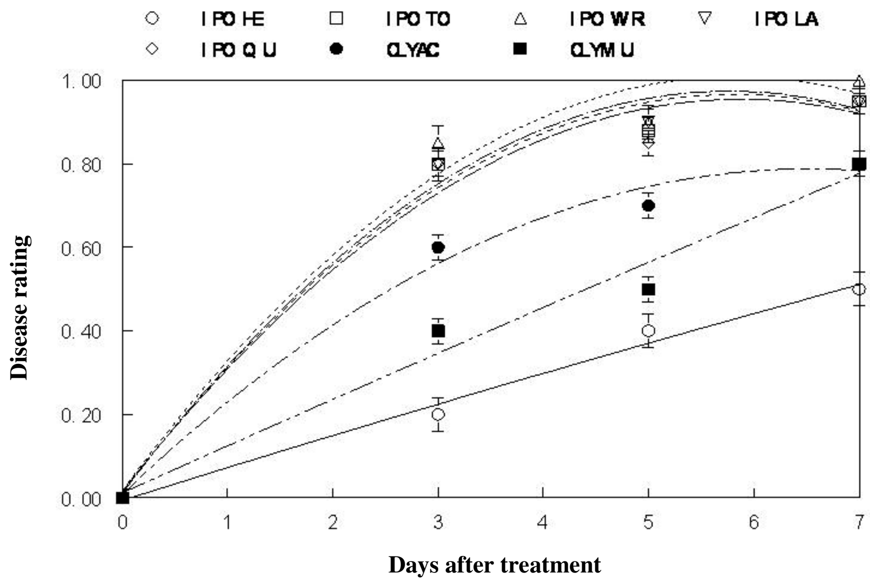

Figure 3. Disease progression of *Myrothecium verrucaria* on several species of morning-glories over a 7- day period. A visual disease severity rating scale modified from Horsfall & Barrett (21), was used to estimate disease progression where: 0 represented unaffected, and 0.2, 0.4, 0.6, 0.8 represent 20, 40, 60, and 80 % leaf and stem lesion coverage/injury, respectively, and 1.0 = plant mortality. The relationships of disease progression are best described by the following equations: IPOHE, $Y = -0.01 + 0.08X$, $R^2 = 0.98$; IPOTO, $Y = 0.16 + 0.32X - 0.03X^2$, $R^2 = 0.98$; IPOWR, $Y = 0.02 + 0.34X - 0.03X^2$, $R^2 = 0.96$; IPOLA, $Y = 0.01 + 0.33X - 0.03X^2$, $R^2 = 0.98$; IPOQU, $Y = 0.02 + 0.32X - 0.03X^2$, $R^2 = 0.98$; CLYAC, $Y = 0.01 + 0.24X - 0.02X^2$, $R^2 = 0.98$; CLYMU, $Y = 0.01 + 0.11X - 0.01X^2$, $R^2 = 0.96$. Error bars represent ± 1 SEM. Species abbreviations: moonflower, CLYAC; ivyleaf, IPOHE; pitted, IPOLA; multicolor, IPOTO; palmleaf, IPOWR; moonvine, CLYMU; cypressvine, IPOQU.

Shoot Length Analysis: Analysis of shoot lengths of these treated plants 7 DAT showed that the MV plus Silwet and MV plus invert emulsion plus Silwet treatments reduced shoot lengths by ca. 60-80 % compared to Silwet alone-treated plants (Fig. 4). The shoot length of some species was reduced by the emulsion alone treatment, i.e., moonflower (CLYAC) (ca. 60%), pitted (IPOLA) (ca. 55%), and multicolor (IPOTO), ivyleaf (IPOHE) and palmleaf (IPOWR) (ca. 25%). In 4 of the 7 morning-glory species, the MV plus invert emulsion plus Silwet treatment tended to reduce shoot lengths more than in the MV plus Silwet treatment, but in 2 species, i.e., cypressvine (IPOQU) and palmleaf (IPOWR), the MV plus invert emulsion plus Silwet treatment caused less length reduction than in the MV plus Silwet treatment (Figure 4). Most of the multicolor (IPOTO) plants in the MV plus invert emulsion plus Silwet treatment succumbed before the termination of the tests at 7DAT, and thus the histogram bar value ranged from only ca. 1-2 % of control (Figure 4.).

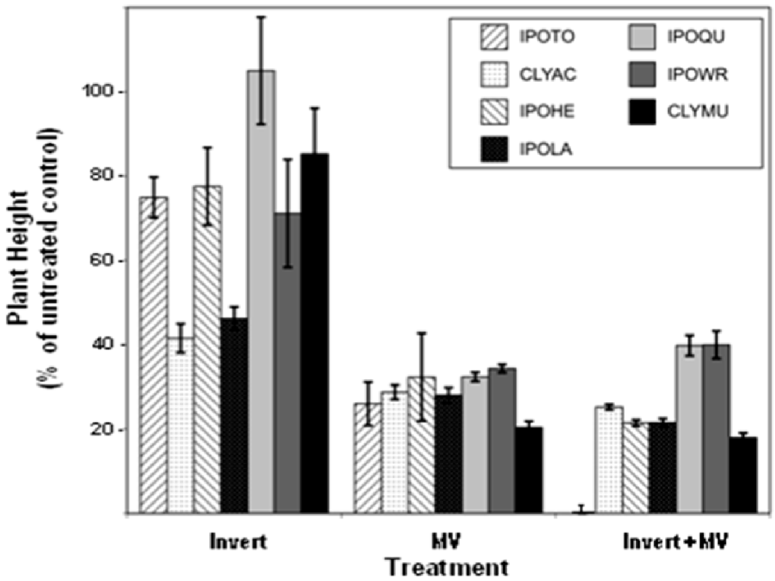

Figure 4. Effects of Silwet, invert emulsion plus Silwet, and MV combined with either, Silwet or invert emulsion plus Silwet, on morning-glory seedling shoot lengths (mm). Species abbreviations: moonflower, CLYAC; ivyleaf, IPOHE; pitted, IPOLA; multicolor, IPOTO; palmleaf, IPOWR; moonvine, CLYMU; cypressvine, IPOQU. Error bars represent ± 1 SEm.

Fresh Weight and Dry Weight Analysis: The invert emulsion plus Silwet treatment caused only a slight reduction (ca. 10-25%) of dry weight accumulation at 7 DAT in these seven morning-glories (Figure 5). However, in the MV plus Silwet treatment of multicolor (IPOTO), moonflower (CLYAC), ivyleaf (IPOHE) and pitted (IPOLA), dry weights were reduced by ca. 69-76 %, and lowered in a range of ca. 43-63 % in cypressvine (IPOQU), palmleaf (IPOWR) and moonvine (CLYMU), compared to their respective untreated controls. Combination of MV plus the invert emulsion resulted in a greater reduction of dry weight accumulation than that caused by the MV alone treatment in two species, IPOTO and IPOLA (Figure 5).

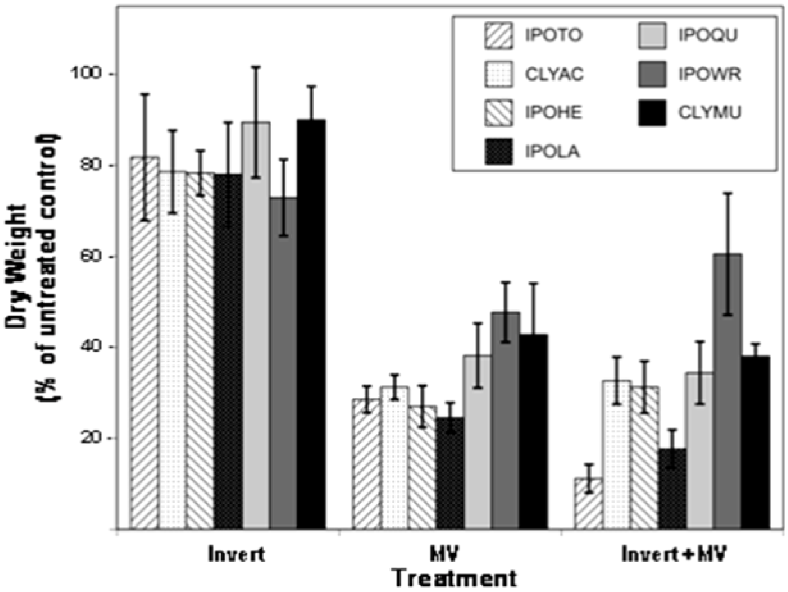

Figure 5. Effects of various treatments on dry weight (g) accumulation of plant shoots of seven morning-glory species, 7 DAT with Silwet, invert emulsion plus Silwet, and MV combined with either, Silwet or invert emulsion plus Silwet. Species abbreviations: moonflower, CLYAC; ivyleaf, IPOHE; pitted, IPOLA; multicolor, IPOTO; palmleaf, IPOWR; moonvine, CLYMU; cypressvine, IPOQU. Error bars represent ± 1 SEM.

Fresh weight accumulation analysis showed that the relative effects of these treatments generally mimicked that of dry weight results with regard to species (data not shown). The similarity in fresh weight and dry weight profiles suggests that none of the treatments caused significant differences in water relations (desiccation or swelling) of these species.

Generally, pitted, multicolor, palmleaf and cypressvine morning-glories were the most susceptible to MV plus Silwet and MV plus invert emulsion plus Silwet treatments (see Figures 1, 3, 4, and 5). Furthermore, mortality of many of the treated plants of these species was evident on, or before 7 DAT when the experiment was terminated. In the more tolerant species, i.e., ivyleaf, moonvine and moonflower morning-glories, severe damage and/or growth reductions were apparent but mortality did not occur at 7 DAT. Treatment of these more tolerant seedlings with MV plus invert emulsion plus Silwet increased efficacy in some cases. This invert emulsion formulation has also been reported to increase the efficacy of other bioherbicides, as well as eliminating or lowering the period of free-moisture or dew required for the spore germination and infection to occur (6,25,26). In the present studies, we did not assess the interactions of MV plus invert emulsion plus Silwet treatment with the length of dew period or with spore germination. Recently we found that MV efficacy can be improved by applying the spores of organism with spray adjuvants or herbicides, when evaluating sicklepod (*Senna obtusifolia* L.)

seedlings as bioassay plants (32). Future studies should test some of these adjuvants and the invert emulsion on mature morningglories under field conditions.

Many of our early studies with MV utilized spore formulations of fungus, but we have recently found that fermentation of MV in submerged culture produces mycelial formulations that are as efficacious as spore formulations, but that are void of, or possess very low amounts of mycotoxins (macrocyclic trichothecenes) (12). In a further extension of our quest to lower or eliminate trichothecene production in this bioherbicide, we discovered that sporulation and macrocyclic trichothecene production could be manipulated by altering the growth media composition (31). Thus, it would also be interesting to examine the effects of invert emulsion on MV mycelia preparations and on spores produced under various cultural conditions.

CONCLUSIONS

Results indicate that MV possesses differential weed control efficacy on morning-glory species and that efficacy can be increased in some species when applied with an invert emulsion in some cases. Shoot length and biomass data (fresh and dry weights) generally paralleled the development and severity of visual injury symptoms, but these assessments were made at 3 and 7 DAT, respectively. We have not tested MV formulations (with or without the invert imulsion) on mature morning-glory plants under field conditions. However, formulations of this bioherbicide are highly efficacious on mature kudzu (*Pueraria lobata*), redvine (*Brunnichia ovata*), trumpetcreeper (*Campsis radicans*) and other weeds when tested in field plots and in natural infestations of these weeds. Thus we suspect that MV in these formulations would also be effective on mature morning-glory species. Preliminary tests in greenhouse on one cultivar of sweet potato (*Ipomoea batatas* Lam.) (cv. 'Beauregard'), using MV in these formulations, indicated that that this cultivar was only slightly affected by inundative inoculations of MV spores applied in an analogous manner as described in the tests reported here on morning-glories.

Data from these present tests expands the knowledge about the bioherbicidal host range of this MV strain, and shows that the bioherbicide can injure and/or cause mortality to a broad range of weeds. Our results also point out that this MV strain (IMI 361690) possesses even a more diverse host range than that of reported for another MV strain that exhibited bioherbicidal activity on morning-glories reported by other researchers.

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