Available online at www.sciencedirect.com
SCIENCE DIRECT®
Crop Protection 25 (2006) 39–46
Crop Protection
www.elsevier.com/locate/cropro

Potential of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi,* as bioherbicides for several weedy *Amaranthus* species

Loretta Ortiz-Ribbing*, Martin M. Williams, II

USDA-ARS, Invasive Weed Management Unit, S-306 Turner Hall, 1102 S. Goodwin Avenue, Urbana, IL 61801, USA

Received 3 February 2005; received in revised form 28 February 2005; accepted 8 March 2005

Abstract

Several plants in the genus *Amaranthus* are weeds in cropping systems throughout the world, and some biotypes have developed resistance to a number of herbicide families. In an effort to develop alternative, biologically based weed management tactics, studies were initiated to quantify the selective ability of two fungal organisms to several *Amaranthus* species. Response of weed seedlings to *Microsphaeropsis amaranthi* (3×10^6 conidia.ml^{-1}), *Phomopsis amaranthicola* (1×10^7 conidia.ml^{-1}), and a mixture of the two organisms ($1.5 \times 10^6 + 4 \times 10^6$ conidia.ml^{-1}, *M. amaranthi* and *P. amaranthicola*, respectively) were tested under controlled and field conditions at Urbana, IL in 2004. Weeds included *Amaranthus rudis*; *A. palmeri*; *A. powellii*; *A. retroflexus*; *A. spinosus*; *A. hybridus*; *A. albus*; and *A. blitoides*. Seeds of each species were sown in the greenhouse, and conidial suspensions were applied at the 2- to 4-leaf stage, and then pots were placed either in a dew chamber (24 h) and back in the greenhouse, or in the inter-row of a soybean field. Treatment with fungal organisms infected most weeds, reducing growth and survival, although responses in the greenhouse were less than those observed in the field. Percent seedling mortality for *A. albus* and *A. blitoides* were between 80 and 100%, 14 to 15 DAT for the mixture or *M. amaranthi* alone, in greenhouse and field trials. In the greenhouse, the mixture of two organisms and *M. amaranthi* alone significantly reduced *A. albus* and *A. blitoides* height. Fungal treatments reduced biomass of *A. powellii*, *A. albus* and *A. blitoides*. In field experiments, all eight weed species treated with *M. amaranthi* or the mixture of both organisms had severe disease ratings 15 DAT, and mortality ranged from 74% to 100%. In addition, these treatments reduced biomass of *A. rudis*, *A. retroflexus*, *A. spinosus*, *A. hybridus*, and *A. albus*. Height of *A. rudis*, *A. hybridus*, and *A. albus* was reduced by all fungal treatments. This research indicates seedlings of several *Amaranthus* species are susceptible to conidial suspensions of *P. amaranthicola* and *M. amaranthi* in both controlled and field environments.

Keywords: Bioherbicide; Mycoherbicide; Biological control; Integrated weed management

1. Introduction

Species of the *Amaranthus* genus are troublesome weeds found growing in many agronomic and horticultural crops throughout the United States (Coetzer et al., 2002; Wax, 1995). In recent years, the prevalence of weedy *Amaranthus* infestations on corn and soybean acreage has increased (Wax, 1995). This has partly resulted from misidentification of the weeds, a shift in tillage and residue-management, and selection for herbicide resistant biotypes within weed populations (Hager et al., 1997). Herbicide resistance world-wide has been reported for *Amaranthus rudis* Sauer., (common waterhemp); *A. palmeri* S. Wats., (Palmer amaranth); *A. powellii* S. Wats., (Powell amaranth); *A. retroflexus* L., (redroot pigweed); *A. spinosus* L., (spiny amaranth); *A. hybridus* L., (smooth pigweed); *A. albus* L., (tumble pigweed); and *A. blitoides* S.Wats., (prostrate pigweed) (Heap, 2000).

Some *Amaranthus* spp. have developed resistance to multiple herbicide families currently used for weed control. Patzoldt et al. (2002) reported *A. rudis* resistant to imidazolinone, sulfonylurea and triazine herbicide

*Corresponding author. Tel.: +1 217 244 6096; fax: +1 217 333 5251
E-mail address: ortizrib@uiuc.edu (L. Ortiz-Ribbing).

0261-2194/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2005.03.021

families. In addition, *A. rudis* populations have been identified in Illinois that exhibit resistance to diphenyl ether herbicides (Shoup et al., 2003), and populations have been identified with resistance to all three herbicide classes (Hartzler, 2003). Furthermore, Patzoldt et al. (2002) found substantial variability in control of *A. rudis* with glyphosate, an herbicide that is used widely since the adoption of glyphosate-tolerant crops. Resistance to several imidazolinone and sulfonylurea herbicides has been reported for *A. hybridus* from locations in Maryland and Virginia (Manley et al., 1996). Compounding the resistance problem is the fact that herbicide resistance can be transferred from a monoecious species such as *A. hybridus* to a dioecious species such as *A. rudis* (Tranel et al., 2002). As the agricultural sector increases its reliance on a select group of herbicides for control of weedy *Amaranthus* species, the potential for the development of resistant weed biotypes is likely to increase.

Alternative, biologically based weed management tactics for *Amaranthus* spp. are being investigated. Researchers at the University of Florida isolated and identified *Phomopsis amaranthicola* Rosskopf, Charudattan, Shabana and Benny, an indigenous plant pathogen that is effective in providing up to 100% control of several *Amaranthus* species (Rosskopf, 1997; Rosskopf et al., 2000a, b). Host range testing of this organism has not shown infection of soybean (*Glycine max* (L.) Merr.), corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), or wheat (*Triticum aestivum* L.); immune plant responses were observed for all 55 plant species representing 17 plant families (Rosskopf, 1997). Mintz et al. (1992) evaluated another fungal pathogen, *Aposphaeria amaranthi* Ell. & Barth., as a potential bioherbicide for *A. albus*. The reported host range for this fungus was restricted to *Amaranthaceae*; the pathogen infected several *Amaranthus* species (*A. retroflexus*, *A. spinosus*, and *A. hybridus*) in addition to *A. albus*. This organism was reclassified (Heiny et al., 1992) in the genus *Microsphaeropsis* and renamed *Microsphaeropsis amaranthi* (Ell. & Barth.). Both *M. amaranthi* and *P. amaranthicola* were isolated from the Southern United States, and little work has been done showing their effectiveness in cropping systems in the Midwest. Smith (2003) illustrated that *M. amaranthi* has potential as an alternative method for managing *A. rudis* in soybean. To date, no research has shown the effectiveness of a mixture of both fungal organisms. Development of alternative weed control methods is needed to help decrease reliance on herbicide use, thus reducing selection pressure for development of herbicide resistance.

The overall goal of this research was to identify the role of *M. amaranthi* and *P. amaranthicola* as new tools for managing weedy *Amaranthus* species. The objective was to quantify the weed suppressive ability of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* to eight different *Amaranthus* species.

2. Materials and Methods

2.1. Experiment background

Cultures of *M. amaranthi* and *P. amaranthicola* were obtained in collaboration with Raghavan Charudattan, University of Florida, Gainesville, FL. Eight species in the genus *Amaranthus*, common to Illinois, were evaluated in this study. Weeds included: *Amaranthus rudis*; *A. palmeri*; *A. powellii*; *A. retroflexus*; *A. spinosus*; *A. hybridus*; *A. albus*; and *A. blitoides*.

2.2. Inoculum preparation

P. amaranthicola and *M. amaranthi* were grown on V8 juice agar using the methods of Rosskopf et al. (2000b). Both organisms were incubated for a period of 14–21 days. Conidia were rinsed from individual plates using a modified method of Mintz et al. (1992) by using 15 ml sterile, distilled water and straining through 2 layers of cheese cloth. Spore suspensions were prepared in 100 ml sterile, distilled water containing 1.0×10^7 conidia ml^{-1} for *P. amaranthicola* and 3.0×10^6 conidia.ml^{-1} for *M. amaranthi*. A hemacytometer (Fuchs Rosenthal counting chamber, Hausser Scientific, Horsham, PA) was used to determine cfu.ml^{-1}. A mixture of both organisms was made with $4.0 \times 10^6 + 1.5 \times 10^6$ conidia.ml^{-1} for *P. amaranthicola* and *M. amaranthi*, respectively. Fungal spore solutions were amended with 0.5% psyllium mucilloid (Metamucil®, Procter and Gamble, Cincinnati, OH). Control solutions were prepared using 100 ml sterile, distilled water, and one was prepared with 100 ml sterile, distilled water plus 0.5% psyllium mucilloid.

2.3. Greenhouse experiment

Ray Leach SC-10 Super Cell "Cone-tainers"™ (cones) (Stuewe & Sons, Inc. Corvallis, OR), with a cell diameter of 3.8 cm, and a cell depth of 21 cm, were used to grow weed seedlings. Seeds of each species were sown in a steam-pasteurized, Torpedo sand:soil:peat (1:1:1) greenhouse mixture. The soil for the mix belonged to the Drummer/Flanagan soil series, and the peat was Canadian sphagnum peat moss. The pH values for the sand and soil were approximately 6.1 and 5.8, respectively. When weeds reached the 2- to 4- true leaf stage they were thinned to two to three seedlings per cone, and arranged in a completely randomized design with 3 replications. Seedlings in the four-leaf stage had four fully expanded true leaves with the fifth leaf beginning to expand and the sixth leaf starting to open (14 days after planting for some species), as described by Mintz et al. (1992).

The day following thinning, conidial suspensions were prepared as described above. Weeds were inoculated

with the fungal organisms using a hand-held, pump spray bottle. Spores suspensions were applied until run-off with approximately 3 ml per plant. Inoculated weed seedlings were incubated in a dark dew chamber with an air temperature of 21°C for 24 hours following inoculation, and then returned to the greenhouse bench for the duration of the experiment. Natural light was supplemented by 1000 W high pressure, sodium vapor lights ($156\,\mu E\,s^{-1}\,m^{-2}$) providing 16 h of daylight. Temperature was maintained at 25 ± 3°C during the day, and 22 ± 3°C for the 8-h dark period. Weed seedlings were watered as necessary to maintain adequate soil moisture.

Disease severity was rated using a modified 0 to 5 scale from Smith (2003): 0 = no visible symptoms, 1 = leaves with small necrotic flecks, but no stem lesions, 2 = discrete lesions on leaves and/or stem, some plant wilting; 3 = lesions >0.5 cm of stem's circumference and leaf tissue with necrotic lesions, more severe wilting of plant and top leaves; 4 = girdling stem lesions and total leaf necrosis, and 5 = plant death or girdled and falling over.

Disease severity and seedling mortality were evaluated at 2, 4, 6, 8, 10, 12 and 14 days following inoculation. Seedling mortality was calculated as a percentage of the number of plants with a disease severity rating of 5 to the total number of treated plants in each replicate. At the termination of each trial, weed height was measured from the soil surface to the apex, and then seedlings were dried for 3 d at 50 C to obtain a constant shoot biomass for surviving plants. The experiment was conducted 4 times.

2.4. Field experiment

Weed seedlings were grown as described above, except flats were used to increase the number of seedlings (3) and replicates (8). The experiment was conducted twice and the data represents two environments. When seedlings reached the 2- to 4- leaf stage, they were inoculated with spore suspensions, containing *M. amaranthi, P. amaranthicola,* or a mixture of the two organisms, prepared and applied in the manner described above. Flats containing the inoculated weed seedlings were placed in the inter-row of a soybean field. The soybean plants created a closed canopy above the weed seedlings. During trial one (23 July–9 August 2004) the soybean plants received sprinkler irrigation for 15 s four times an hour during the daytime and 15 s every hour during the night. The sprinkles were shut off for the duration of the second trial (24 August–8 September 2004); however, the field received natural precipitation during the first 6 days of the 2-week period (Table 1). Disease severity was rated using the above scale at 3, 5, 7, 10, 12 and 15 d after inoculation. Percent seedling mortality, plant height and shoot biomass were determined at the end of each trial as described above.

2.5. Statistical analysis

All data were subjected to analysis of variance and covariance using the mixed models procedure of SAS (Release 8.2. SAS Institute, 2001, Cary, NC). Inoculation treatment was the fixed main effect for each weed species. Repeated trials and the trial by treatment interaction were designated as random effects. Seedling mortality and shoot biomass were transformed using the arc-sine square-root transformation prior to analysis to equalize variance. Non-transformed means are reported with transformed *P*-value. Multiple comparisons on the differences of least squares-means were done using Dunnett at $P = 0.05$. Least significant differences (LSD, $P = 0.05$ level) were calculated from the standard

Table 1
Precipitation and average daily temperature in Champaign-Urbana, IL during experiment period (23 July–14 September 2004) and 29-year monthly average

		Precipitation (cm)		Temperature (°C)	
Month	Dates	Weekly total	29-Year average	Average daily	29-Year average
July	23–31	1.2	—	21	—
	Monthly total	14.6	11.9	23	24
August	1–7	0.38	—	21	—
	8–14	2.2	—	17–18	—
	15–21	1.2	—	19	—
	22–31	5.3	—	22	—
	Monthly total	9.1	11.1	20	23
September	1–7	0	—	23	—
	8–14	0	—	21	—
	Monthly total	5.6	8.2	21	19

error of differences (SEDs) for pairs of means multiplied by the student t value.

3. Results and Discussion

3.1. Greenhouse experiment

In greenhouse experiments shoot biomass and height reductions, as well as high disease severity ratings and seedling mortality were observed on many of the weedy *Amaranthus* species after application with either of the organisms alone or with the mixture of both organisms. The fungal treatments significantly reduced shoot biomass of *A. palmeri* , *A. albus*, and *A. blitoides* relative to the control (Table 2). *Amaranthus hybridus* seedlings treated with the mixture of both organisms and with *P. amaranthicola* had lower ($P<0.09$) biomass than *A. hybridus* treated with the *M. amaranthi* treatment alone. Most seedlings had lower biomass values compared to the control, but differences were not significant. The mixture reduced biomass of all weed species; no significant differences were observed among the eight species. However, each fungal organism alone showed significant differences in biomass of surviving plants among the eight species, indicating a less consistent effect. Shoot biomass for *A. rudis* was not significantly reduced in greenhouse experiments. Smith (2003) reported no significant reductions in *A. rudis* shoot biomass with *M. amaranthi*, but a trend showed greater biomass reductions when 3- to 4-leaf stage *A. rudis* seedlings were treated.

Significant differences in percent seedling mortality between the fungal treatments were only observed for *A. albus* and *A. blitoides* (Table 2). Percent seedling mortality was between 80% and 92% by 14 days after treatment (DAT) with either the mixture or with *M. amaranthi* alone. Percent seedling mortality of *A. powellii* from the *P. amaranthicola* treatment was greater ($P<0.10$) than the other fungal treatments. None of the fungal treatments caused seedling mortality of *A. spinosus* under greenhouse conditions. Significant difference in seedling mortality among the eight weed species were observed for each fungal treatment.

The fungal treatments caused significantly greater visual symptoms of disease on all eight species compared to the untreated weeds (Table 3). The highest visual ratings for disease symptoms which included foliar and stem lesions, were seen on *A. albus* and *A. blitoides*. The highest ratings for disease on *A. powellii* and *A. spinosus* were achieved by treating with *P. amaranthicola* alone. Disease severity ratings were significantly higher with the mixture on *A. powellii* and *A. spinosus* ($P<0.09$) compared to the application of *M. amaranthi* alone, but not compared to *P. amaranthicola* alone. Including psyllium mucilloid in the inoculum mixture as a humectant had no effect on seedling disease symptom severity (data not presented).

Differences among species in seedling mortality and disease severity could have resulted because both fungi produced foliar lesions on some species without lesion development on the seedling's stem. Both fungi caused leaf-spotting symptoms that often resulted in leaf senescence, on all eight weed species, however, seedlings frequently survived by producing new leaves. Our observations, concur with Rosskopf (1997) and Smith (2003); infection and lesion development on both leaf and stem tissue, leading to stem girdling, was important

Table 2
Shoot biomass and seedling mortality for eight *Amaranthus* species 14 days after treatment with *M. amaranthi*, *P. amaranthicola* or a mixture of both organisms

	Shoot biomass of surviving plant (mg per plant)[a]					Percent seedling mortality				
Weed species	Control[b]	Mixture	*M. amaranthi*	*P. amaranthicola*	Pr > *F*[c]	Control	Mixture	*M. amaranthi*	*P. amaranthicola*	Pr > *F*
A. rudis	97.3 a[d]	59.7 a	88.9 a	75.7 a	0.63	0 a	33 a	4 a	0 a	0.12
A. palmeri	112.4 a	68.8 b	83.2 b	68.5 b	<0.01	0 a	19 a	8 a	13 a	0.31
A. powellii	84.5 a	56.9 a	84.7 a	46.2 a	0.17	0 a	0 a	4 a	29 a	0.10
A. retroflexus	73.2[a]	47.1 a	77.6 a	50.5 a	0.19	0 a	10 a	8 a	21 a	0.31
A. spinosus	81.2 a	41 a	68.3 a	50.1 a	0.15	0 a	0 a	0 a	0 a	1.00
A. hybridus	66.7 a	34.2 a	62.1 a	32.0 a	0.09	0 a	0 a	8 a	38 a	0.18
A. albus	98.3 a	5.1 b	3.9 b	22.5 b	<0.01	0 a	92 c	88 c	42 b	<0.01
A. blitoides	71.2 a	11.9 bc	28.3 bc	32.3 b	0.03	0 a	80 c	82 c	34 b	<0.01
LSD (0.05)	n.s.[e]	n.s.	39.0	25.2	—	n.s.	47	17	28	—

[a]Shoot biomass and seedling mortality are the average of results from 4 greenhouse trials. Non-transformed means are presented with transformed *P*-value.

[b]Treatments consist of a water control, the mixture of *P. amaranthicola* and *M. amaranthi* ($4.0\times10^6+1.5\times10^6$ conidia.ml^{-1}, respectively), *M. amaranthi* alone (3.0×10^6 conidia.ml^{-1}) and *P. amaranthicola* alone (1.0×10^7 conidia.ml^{-1}).

[c]Pr > *F* value for testing the hypothesis that the fungal treatments are different.

[d]Numbers followed by the same letter within a row are not significantly different at $P<0.05$.

[e]n.s. = not significant at $P<0.05$.

Table 3
Disease severity ratings and weed height for eight *Amaranthus* species 14 days after treatment with *M. amaranthi*, *P. amaranthicola* or a mixture of both organisms

	Disease severity rating[a]					Weed height (mm)[b]				
Weed species	Control[c]	Mixture	*M. amaranthi*	*P. amaranthicola*	Pr > *F*[d]	Control	Mixture	*M. amaranthi*	*P. amaranthicola*	Pr > *F*
A. rudis	0.0 a[e]	3.4 b	2.1 b	1.2 ab	0.04	57.1 a	31.0 a	57.2 a	64.9 a	0.22
A. palmeri	0.0 a	2.8 c	1.5 b	2.1 bc	<0.01	40.2 a	29.0 a	37.3 a	39.9 a	0.21
A. powellii	0.0 a	1.4 ab	1.1 a	2.8 b	0.01	52.5 a	56.7 a	53.1 a	47.6 a	0.48
A. retroflexus	0.0 a	2.9 b	1.7 b	3.0 b	0.01	36.8 a	29.6 a	37.6 a	33.1 a	0.73
A. spinosus	0.0 a	2.1 bc	1.2 b	2.2 c	<0.01	32.6 a	27.6 a	34.3 a	32.8 a	0.49
A. hybridus	0.0 a	2.3 b	2.5 b	3.0 b	0.02	40.9 a	36.4 a	41.7 a	29.4 a	0.72
A. albus	0.0 a	4.8 b	4.8 b	3.8 b	<0.01	40.4 a	1.5 b	5.7 b	21.2 ab	0.03
A. blitoides	0.0 a	4.3 bc	4.6 c	2.9 b	<0.01	43.7 a	5.1 b	15.0 b	29.2 ab	0.04
LSD (0.05)	n.s.[f]	1.42	0.66	1.0	—	10.0	n.s.	11.0	15.0	—

[a]Disease severity was rated on a 0–5 scale, where 0 = healthy and 5 = dead weeds. Disease severity is the average of results from 4 greenhouse trials.
[b]Weed height is the average of results from three greenhouse trials.
[c]Treatments consist of a water control, the mixture of *P. amaranthicola* and *M. amaranthi* ($4.0 \times 10^6 + 1.5 \times 10^6$ conidia.ml^{-1}, respectively), *M. amaranthi* alone (3.0×10^6 conidia.ml^{-1}) and *P. amaranthicola* alone (1.0×10^7 conidia.ml^{-1}).
[d]Pr > *F* value for testing the hypothesis that the fungal treatments are different.
[e]Numbers followed by the same letter within a row are not significantly different at $P<0.05$.
[f]n.s. = not significant at $P<0.05$.

for seedling mortality. Wyss and Charudattan (2000) have illustrated that *P. amaranthicola* spore attachment, germination and infection is highly host-specific between two *Amaranthus* species. Differences in spore attachment, germination and infection of these organisms on various *Amaranthus* species could explain mortality differences among species.

The mixture of both organisms and *M. amaranthi* alone significantly reduced height of *A. albus* and *A. blitoides* compared to control seedlings (Table 3). Height of *A. albus* seedlings treated with *P. amaranthicola* was lower than control plants at the 7% level. The mixture of both organisms reduced height of many seedlings compared to the control and to each fungal organism alone, but the reduction was not statistically significant. The height of *A. albus* and *A. blitoides* seedlings was lower ($P<0.07$, $P<0.06$, respectively) after treatment with the mixture of both organisms than after treatment with *P. amaranthicola* alone. As with biomass, the mixture consistently reduced height of all weed species, and no significant differences were observed among the eight species. However, treatment with each fungal organism alone showed significant differences in height among the eight species, indicating a less consistent effect.

3.2. Field experiment

The mixture of both organisms and *M. amaranthi* alone caused significant reductions in shoot biomass relative to the control for five out of eight weed species (Table 4). Treatment with *P. amaranthicola* alone significantly reduced shoot biomass relative to the control for *A. hybridus* and *A. albus*, while reductions of *A. rudis* were significant at ($P<0.06$).

Percent seedling mortality ranged from 74% to 100% for all eight weed species treated with the mixture of the two organisms or with *M. amaranthi* alone (Table 4). *P. amaranthicola* resulted in seedling mortality only for *A. albus*. Seedling mortality for *A. blitoides* caused by *P. amaranthicola* was greater than the control ($P<0.11$).

All eight weed species treated with *M. amaranthi* or the mixture of the two organisms had disease severity ratings of 4.4 to 5 after 15 days (Table 5). In addition, height of all weed species, except *A. palmeri* and *A. blitoides*, was significantly reduced by the mixture of both organisms and by *M. amaranthi* alone. A significant reduction in weed height from *P. amaranthicola* alone was observed for *A. rudis*, *A. hybridus*, and *A. albus*.

In field experiments, weed species had a greater response to the fungal treatments with respect to disease severity, seedling mortality, and weed height and biomass reduction compared to greenhouse results. Typically, bioherbicides that possess good activity under greenhouse conditions fail in the field, because a prolonged period of moisture necessary for infection is lacking under field conditions (Rosskopf et al., 1999). However, field conditions for both trials in this study were provided with optimum moisture either by mist irrigation or natural rainfall (Table 1), that enhanced the humid environment provided by the soybean canopy. In the greenhouse, humid conditions lasted only during the initial 24 h dew period, afterwards plants placed on the greenhouse bench received moisture only from overhead watering. Increased seedling response in the field could

Table 4
Shoot biomass and seedling mortality for eight *Amaranthus* species 15 days after treatment with *M. amaranthi*, *P. amaranthicola* or a mixture of both organisms

	Shoot biomass of surviving plant (mg per plant)[a]					Percent seedling mortality				
Weed species	Control[b]	Mixture	*M. amaranthi*	*P. amaranthicola*	$Pr > F$[c]	Control	Mixture	*M. amaranthi*	*P. amaranthicola*	$Pr > F$
A. rudis	15.7 a[d]	0 b	0.69 b	13.0 a	<0.001	2 a	100 b	97 b	22 a	<0.01
A. palmeri	17.9 a	4.5 a	4.0 a	22.2 a	0.12	9 a	83 b	81 b	23 a	0.05
A. powellii	18.5 a	4.2 a	6.9 a	17.2 a	0.24	2 a	81 b	74 b	18 a	0.03
A. retroflexus	15.3 a	1.7 b	0.99 b	10.1 a	<0.01	4 a	87 b	93 b	16 a	0.01
A. spinosus	13.3 a	0.0 b	2.1 b	9.0 a	<0.01	0 a	100 b	98 b	0 a	<0.01
A. hybridus	18.9 a	0.0 c	1.4 c	9.6 b	<0.001	4 a	100 b	98 b	11 a	<0.01
A. albus	14.0 a	0.0 c	0.0 c	2.1 b	<0.001	18 a	100 b	100 b	83 b	0.04
A. blitoides	19.1 a	0.54 a	0.0 a	4.8 a	0.15	15 a	100 b	100 b	46 a	0.01
LSD (0.05)	n.s.[e]	n.s.	n.s.	10.1	—	n.s.	n.s.	n.s.	33	—

[a]Shoot biomass and seedling mortality are the average of results from 2 field trials. Non-transformed means are reported with transformed *P*-value.
[b]Treatments consist of a water control, the mixture of *P. amaranthicola* and *M. amaranthi* ($4.0 \times 10^6 + 1.5 \times 10^6$ conidia.ml^{-1}, respectively), *M. amaranthi* alone (3.0×10^6 conidia.ml^{-1}) and *P. amaranthicola* alone (1.0×10^7 conidia.ml^{-1}).
[c]$Pr > F$ value for testing the hypothesis that the fungal treatments are different.
[d]Numbers followed by the same letter within a row are not significantly different at $P < 0.05$.
[e]n.s. = not significant at $P < 0.05$.

Table 5
Disease severity ratings and weed height for eight *Amaranthus* species 15 days after treatment with *M. amaranthi*, *P. amaranthicola* or a mixture of both organisms

	Disease severity rating[a]					Weed height (mm)				
Weed species	Control[b]	Mixture	*M. amaranthi*	*P. amaranthicola*	$Pr > F$[c]	Control	Mixture	*M. amaranthi*	*P. amaranthicola*	$Pr > F$
A. rudis	0.1 a[d]	5.0 c	4.9 c	2.5 b	<0.01	62.8 a	0.0 c	0.88 c	39.7 b	<0.001
A. palmeri	0.5 a	4.6 b	4.4 b	2.0 a	0.01	35.1 a	4.7 a	5.6 a	37.2 a	0.06
A. powellii	0.2 a	4.7 c	4.4 c	2.2 b	<0.01	57.8 a	6.3 b	10.4 b	41.7 a	0.02
A. retroflexus	0.22 a	4.8 c	4.8 c	2.6 b	<0.01	39.9 a	4.3 b	1.9 b	30.1 a	<0.01
A. spinosus	0.0 a	5.0 c	5.0 c	1.8 b	<0.01	28.5 a	0.0 b	0.63 b	25.2 a	0.03
A. hybridus	0.4 a	5.0 c	4.9 c	2.4 b	<0.01	47.4 a	0.0 c	0.87 c	36.5 b	<0.001
A. albus	1.1 a	5.0 b	5.0 b	4.5 b	0.03	39.6 a	0.0 b	0.0 b	6.3 b	<0.01
A. blitoides	1.8 a	5.0 c	5.0 c	3.3 b	<0.01	43.2 a	1.6 a	0.0 a	19.8 a	0.08
LSD (0.05)	n.s.[e]	n.s.	n.s.	0.59	—	n.s.	n.s.	n.s.	14.5	—

[a]Disease severity was rated on a 0–5 scale, where 0 = healthy and 5 = dead weeds. Disease severity and weed height are the average of results from 2 field trials.
[b]Treatments consist of a water control, the mixture of *P. amaranthicola* and *M. amaranthi* ($4.0 \times 10^6 + 1.5 \times 10^6$ conidia.ml^{-1}, respectively), *M. amaranthi* alone (3.0×10^6 conidia.ml^{-1}) and *P. amaranthicola* alone (1.0×10^7 conidia.ml^{-1}).
[c]$Pr > F$ value for testing the hypothesis that the fungal treatments are different.
[d]Numbers followed by the same letter within a row are not significantly different at $P < 0.05$.
[e]n.s. = not significant at $P < 0.05$.

have resulted because seedlings grown under greenhouse conditions and moved into the field typically tend to be more susceptible to abiotic factors. Therefore, follow-up field research is needed investigating the efficacy of these fungal organisms on weeds grown entirely under field conditions. Other research such as Mintz et al. (1992) illustrated a successful transition between greenhouse and field efficacy for *M. amaranthi*, although the study represented one season at one location. Rosskopf et al. (2000b) confirmed greenhouse results with field efficacy trials evaluating *P. amaranthicola* mortality on several *Amaranthus* spp. in the field over a three year period. The current field study represents one location with two different environments created by moisture regimes that produced similar results. This may indicate the usefulness of these fungal pathogens for *Amaranthus* control in humid cropping systems or those utilizing overheard irrigation.

The primary benefit of using a mixture of fungal organisms for weed management is two-fold; a blend of pathogens can increase the number of target weed species, and several pathogens for a single weed species may improve efficacy and insure against possible failure of either pathogen (Chandramohan and Charudattan, 2003). In this study, the mixture helped increase the number of target species compared to *P. amaranthicola* alone. This may have resulted because each organism has different optimal temperatures; therefore, infection is more likely with both organisms compared to a single organism. Temperature, duration of surface wetness, and rain were reported as being the most important environmental factors for disease development of *Phomopsis* (Rupe and Ferris, 1987). Rosskopf (1997) reported that dew period temperatures below 20°C significantly decreased *P. amaranthicola* efficacy. Dew period temperature also has been shown to limit the efficacy of *M. amaranthi*. Mintz et al. (1992) reported an optimal temperature range of between 20 °C and 28 °C for 100% mortality of *A. albus*. Smith (2003) showed maximum *M. amaranthi* conidia germination at 20 °C and severely reduced germination below 15 °C or above 25 °C. The mixture of both organisms and *M. amaranthi* alone provided significantly greater disease severity, seedling mortality, and reduced shoot biomass and seedling height compared to *P. amaranthicola* alone for many of the weeds except *A. albus* and *A. blitoides*. This is explained in part because daily temperatures during the field portion of this experiment were below normal, and corresponded more closely with the optimum temperature range reported for *M. amaranthi*.

Bioherbicides need to be evaluated within integrated weed management systems. Research has demonstrated that chemical herbicides may provide a nutrient source for some microorganisms, and that herbicides can predispose plants to infection from fungal plant pathogens (Rosskopf et al., 1999; Smith, 2003). An increased susceptibility of *Cassia obtusifolia* L. to a fungal bioherbicide, *Alternaria cassiae* Jurair and Khan., was observed when applied in combination with glyphosate (Sharon et al., 1992). Leger et al. (2001) indicated a similar response to glyphosate when mixed with a fungal pathogen for control of fireweed (*Epilobium angustifolium* L.). Smith (2003) evaluated *A. rudis* control and the potential for combining *M. amaranthi* with glyphosate. He found that tank mixing glyphosate with *M. amaranthi* and split-applications of glyphosate followed by *M. amaranthi* 1 and 3 d afterwards, predisposed *A. rudis* to infection by *M. amaranthi*, resulting in reduced weed dry weight and increased weed mortality compared to either *M. amaranthi* or glyphosate alone. However, when *M. amaranthi* was applied first, a reduction in the activity of glyphosate was observed. Fungal pathogens have also shown promise for weed management when combined with interspecific competition strategies from *Trifolium pretense* L.(Guntli et al., 1999), and *Paspalum notatum* Fluegge var. *saurae* Parodi (Yandoc et al., 2004). The selectivity of fungal pathogens makes them appropriate in integrated weed management systems where a single weed species predominates (Guntli et al., 1999). In this study, while *P. amaranthicola* or *M. amaranthi* provided high seedling mortality of some species, their role in integrated weed management systems may involve the pathogen's suppressive effects on weed growth, and subsequent interaction with succeeding weed management tactics.

4. Conclusions

This study indicates seedlings of several *Amaranthus* species are susceptible to conidial suspensions of *P. amaranthicola* and *M. amaranthi* in both controlled and field environments, and that they could potentially provide an alternative biologically based method for managing weeds in the genus *Amaranthus*. Benefits of using a mixture of both organisms, relative to a single organism include activity on more weedy *Amaranthus* species, and a greater likelihood of infection of sensitive species under variable air temperatures.

Acknowledgements

We thank Raghaven Charudattan for his assistance in obtaining cultures of these organisms, and Ruth Green and her staff for their assistance in the greenhouse. Without the assistance of Megan Cook, Ryan Hasty, and Jim DeValerio this research would not have been possible.

References

Chandramohan, S., Charudattan, R., 2003. A multiple-pathogen system for bioherbicidal control of several weeds. Biocontrol Sci. Technol. 13, 199–205.

Coetzer, E., Al-Khatib, K., Peterson, D.E., 2002. Glufosinate efficacy on *Amaranthus* species in glufosinate-resistant soybean (*Glycine max*). Weed Technol. 16, 326–331.

Guntli, D., Burgos, S., Kump, I., Heeb, M., Pfirter, H.A., Défago, G., 1999. Biological control of hedge bindweed (*Calystegia sepium*) with *Stagonospora convolvuli* strain LA39 in combination with competition from red clover (*Trifolium pretense*). Biol. Control 15, 252–258.

Hager, A.G., Wax, L.M., Simmons, F.W., Stoller, E.W., 1997. Waterhemp management in agronomic crops. Bull X855, Information Services, College of Agricultural, Consumer, and Environmental Sciences, University of Illinois, Urbana, IL pp. 4–5.

Hartzler, R.G., 2003. Waterhemp—the perfect weed? North American Weed Management Association Invasive Plant Species Workshop, Kansas City, February 12-13. http://www.weeds.iastate.edu/mgmt/2003/symposium.shtml. Weed Sci. Soc. Am.

Heap, I., 2000. International Survey of Herbicide Resistant Weeds. Web page: www.weedscience.com. Accessed: September, 2004.

Heiny, D.K., Mintz, A.S., Weidemann, G.J., 1992. Redisposition of *Aposphaeria amaranth* in *Microsphaeropsis*. Mycotaxon 44, 137–154.

Leger, C., Hallett, S.G., Watson, A.K., 2001. Performance of *Colletotrichum dematium* for the control of fireweed (*Epilobium angustifolium*) improved with formulation. Weed Technol. 15, 437–446.

Manley, B.S., Wilson, H.P., Hines, T.E., 1996. Smooth pigweed (*Amaranthus hybridus*) and livid amaranth (*A. lividus*) response to several imidazolinone and sulfonylurea herbicides. Weed Technol. 10, 835–841.

Mintz, A.S., Heiny, D.K., Weidemann, G.J., 1992. Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. Plant Dis. 76, 267–269.

Patzoldt, W.L., Tranel, P.J., Hager, A.G., 2002. Variable herbicide responses among Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) populations. Crop Prot. 21, 707–712.

Rosskopf, E.N., 1997. Evaluation of *Phomopsis amaranthicola* sp. nov. as a biological control agent for *Amaranthus* spp. Ph.D. Dissertation, University of FL, Gainesville, FL, USA.

Rosskopf, E.N., Charudattan, R., Kadir, J.B., 1999. Use of plant pathogens in weed control. In: Bellows, T.S., Fisher, T.W. (Eds.), Handbook of Biological Control: Principles and Applications. Academic Press, San Diego, pp. 891–918.

Rosskopf, E.N., Charudattan, R., Shabana, Y.M., Benny, G.L., 2000a. Phomopsis amaranthicola. Mycologia 92, 114–122.

Rosskopf, E.N., Charudattan, R., DeValerio, J.T., Stall, V.M., 2000b. Field evaluation of *Phomopsis amaranthicola*, a biological control agent of *Amaranthus* spp. Plant Dis. 84, 1225–1230.

Rupe, J.C., Ferris, R.S., 1987. A model for predicting the effects of microclimate on infection of soybean by *Phomopsis longicolla*. Phytopathology 77, 1162–1166.

SAS Institute, 2001. The SAS system for Windows. Release 8.2. SAS Institute, Cary, NC.

Sharon, A., Amsellem, Z., Gressel, J., 1992. Glyphosate suppression of an elicited defense response: increased susceptibility of *Cassia obtusifolia* to a mycoherbicide. Plant Physiol. 98, 654–659.

Shoup, D.E., Al-Katib, K., Peterson, D.E., 2003. Common waterhemp (*Amaranthus rudis*) resistance to protoporphyrinogen oxidase-inhibiting herbicides. Weed Sci. 51, 145–150.

Smith, D.A., 2003. Evaluation of *Microsphaeropsis amaranthi* as a bioherbicide for the control of waterhemp (*Amaranthus tuberculatus*). M.S. Thesis, Purdue University, West Lafayette, IN. 47907. USA.

Tranel, P.J., Wassom, J.J., Jeschke, M.R., Rayburn, A.L., 2002. Transmission of herbicide resistance from a monoecious to a dioecious weedy *Amaranthus* species. Theor. Appl. Genet. 105, 674–679.

Yandoc, C.B., Charudattan, R., Shilling, D.G., 2004. Suppression of cogongrass (*Imperata cylindrical*) by a bioherbicidal fungus and plant competition. Weed Sci. 52, 649–653.

Wax, L.M., 1995. Pigweeds of the Midwest—distribution, importance and management. Proceedings of the Iowa Integrated Crop Management Conference, Vol. 7, pp. 239–242.

Wyss, G.S., Charudattan, R., 2000. Infection process of *Phomopsis amaranthicola* on *Amaranthus* spp. and implications for their biological control. In: Alfoldi, T., Lockeretz, W. (Eds.), The World Grows Organic. Proceedings of the 13th International IFOAM Scientific Conference, 28–31 August 2000, Basel, Switzerland, p. 187.