Performance of Two Bioherbicide Fungi for Waterhemp and Pigweed Control in Pumpkin and Soybean

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Abstract

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Common waterhemp (*Amaranthus rudis*) and pigweeds (*Amaranthus* spp.) are troublesome weeds in many cropping systems and have evolved resistance to several herbicides. Field trials to further develop *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* as bioherbicides for control of waterhemp and pigweeds were conducted to test the effectiveness of these organisms in irrigated and nonirrigated pumpkin and soybean plots over 2 years at three locations in western Illinois. The bioherbicide was applied with lecithin and vegetable oil at 187 liters ha⁻¹ in 2008 and 374 liters ha⁻¹ in 2009. Treatments included spore suspensions of *M. amaranthi* and *P. amaranthicola* alone, a mixture of both organisms, and sequential treatments of the organisms

with halosulfuron-methyl (Sanda Herbicide) in pumpkin or glyphosate (Roundup Original Max Herbicide) in soybean. Bioherbicide effectiveness was estimated at approximately 7 and 14 days after treatment, as disease incidence, disease severity, percent weed control, and weed biomass reduction. Significant reductions in weed biomass occurred in treatments with one or both of the fungal organisms, and potential exists to tank mix *M. amaranthi* with halosulfuron-methyl. Leaf surface moisture and air temperatures following application may account for inconsistencies in field results between year and locations. These fungal organisms show potential as bioherbicides for weeds in the genus *Amaranthus*.

The increase in herbicide resistance among weed populations creates challenges to crop production. Recently in the Midwest, populations of common waterhemp (*Amaranthus rudis* Sauer) and pigweeds (*Amaranthus* spp.) have evolved resistance to several herbicide families (5,10,17,18). As weed management practices move toward less tillage and using select groups of herbicides with less soil residual activity, the number of resistant weed biotypes continues to rise and the prevalence of common waterhemp and pigweeds has increased (12,33). Common waterhemp can establish dense infestations in fields repeatedly treated with the same herbicides. It produces large numbers of seed with prolonged dormancy and an extended period of germination through the spring and summer months (9). Waterhemp has replaced redroot pigweed (*A. retroflexus* L.) as the number one weed in vegetable crops, including pumpkin (*Cucurbita pepo*; 12). Researchers in Missouri, Ohio, and, most recently, Illinois have described populations of waterhemp not controlled by normal field-use rates of glyphosate (Roundup Original Max Herbicide; Monsanto Company, St. Louis) in soybean (*Glycine max*; 5,18). In Illinois alone, nearly three-quarters of common waterhemp populations are resistant to ALS-inhibitor herbicides and farmers are advised not to use them for controlling this weed (5–7). In addition, waterhemp and pigweeds have evolved resistance to several herbicide families currently used in soybean (9,17,18,24) and resistance is expected to increase on pumpkin acres as tillage and weeding decrease and the use of herbicides with shorter residuals increases (12). There is a need for

alternative methods for controlling waterhemp and pigweeds resistant to glyphosate or the ALS-inhibitor, halosulfuron-methyl (Sanda Herbicide; Gowan Company, L.L.C., Yuma, AZ) in soybean and pumpkin crops.

The bioherbicide approach has shown promise for control of herbicide-resistant weeds in production agriculture and is an attractive and appropriate choice for growers because bioherbicides can be applied with conventional herbicide application equipment and used in a manner similar to postemergence herbicides (3,4,8). In addition, most fungal organisms introduced using either classical or inundative biocontrol methods have an excellent safety record regarding nontarget damage (1).

Phomopsis amaranthicola Roskopf, Charud., Shabana, & Benny and *Microsphaeropsis amaranthi* Heiny & Mintz (Ell. & Barth.) are indigenous plant pathogens isolated from *Amaranthus* spp. in the southeastern United States (11,13,21). Both organisms have been shown to provide excellent control of several pigweed species in controlled environment and greenhouse trials, and in a small number of field trials (15,16,19–23,25–32,34,35). Host range testing of both organisms has shown that they are specific to the genus *Amaranthus* (13,19) and that they are virulent against a number of weedy species in this genus (13,15,20). In addition, benefits of using a mixture of both organisms, relative to a single organism, may include activity on more weedy *Amaranthus* spp. and a greater likelihood of infection under variable environmental conditions (16).

Ortiz-Ribbing and Williams (15) treated eight weedy *Amaranthus* spp. with either *M. amaranthi*, *P. amaranthicola*, or mixtures of the two, and found that both fungi caused severe disease and mortality ranging from 74 to 100%. In addition, at field locations in Indiana and at our Macomb location, Singh et al. (26) found a synergistic interaction between *M. amaranthi* and glyphosate that enhanced disease severity (DS) and weed control in soybean. Greater than 90% weed control was seen in treatments where both *M. amaranthi* and glyphosate were used (25). Although glyphosate provided good weed suppression, weed escapes were common; however, combination applications of glyphosate with *M. amaranthi* provided excellent control (25). This demonstrated the strong potential of the bioherbicide to be integrated into soybean production, particularly where control by glyphosate is inadequate. In

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addition, *M. amaranthi* was applied as a bioherbicide to control common waterhemp and redroot pigweed populations in overhead- and drip-irrigated processing tomato. Efficacy was good, and high levels of weed suppression were observed when the bioherbicide was applied to small weeds (27).

Unless specially formulated, the survival, infectivity, and impact of fungal pathogens used as bioherbicides will be constrained by the environment. Moran and Showler (14) found limited efficacy with these organisms in the low humidity and high temperature environment of southern Texas. Irrigation is one method to increase plant growth under dry conditions. It may be possible to increase disease incidence and severity under irrigated conditions, which could augment humidity and moisture on the plant leaf surfaces, thus providing a beneficial environment for these fungal bioherbicide organisms to infect and kill *Amaranthus* weeds.

In addition, acceptance of these bioherbicides may be greater if they fit into current weed management practices. Smith and Hallett (31) and Wyss et al. (35) found that glyphosate reduced spore germination of *M. amaranthi* and *P. amaranthicola*, respectively. Therefore, it is better to use sequential treatments where glyphosate is followed by the bioherbicide organism. However, the effect of halosulfuron-methyl, a common herbicide used in pumpkin production, on these organisms is unknown.

This research evaluates a method for integrating these bioherbicide organisms into production systems along with other weed management technologies. The objectives of this research were to (i) further develop two native fungal pathogens (*M. amaranthi* and *P. amaranthicola*) as bioherbicides for control of common waterhemp and pigweeds by improving our understanding of their potential to be integrated into weed management systems in irrigated and nonirrigated pumpkin and soybean crops; (ii) determine whether these organisms are effective in the field alone, as a mixture, or as sequential treatments with either glyphosate or halosulfuron-methyl; and (iii) evaluate the potential of these organisms as tank mix partners with halosulfuron-methyl. The development of alternative, biologically based weed control technologies that can be integrated into weed management systems for these troublesome waterhemp and pigweeds will help reduce the selection pressure for herbicide resistance in weed populations and extend the useful life of popular herbicides.

Materials and Methods

Cultures of *M. amaranthi* (isolate GJW-AR1) and *P. amaranthicola* (GJW-RC isolate B) were obtained from Dr. R. Charudattan, University of Florida Gainesville under United States Department of Agriculture–Animal and Plant Health Inspection Services PPQ permits 00299 and 00032. Cultures were received and maintained for long-term storage in sterilized soil in sterile glass tubes in the dark at 4°C. The soil was a SB300 Universal Mix, 60% by weight mixed with 40% fine silica sand.

Herbicide compatibility study. Preliminary laboratory experiments tested radial growth inhibition and conidia production of the two bioherbicide fungi with halosulfuron-methyl (Sanda Herbicide; Gowan Company). *M. amaranthi* and *P. amaranthicola* were grown on V8 juice agar using the methods of Roskopf et al. (20) and Ortiz-Ribbing and Williams (15,16). Sandea Herbicide, consisting of 75% active ingredient, was added to sterilized V8 liquid media at five concentrations (0, 250, 380, 500, and 1,000 µg ml⁻¹); 250 to 500 µg/ml represents the range of recommended rates of formulated product for halosulfuron-methyl.

One 5-mm-diameter agar plug, made with a sterile cork borer, was taken from a 14-day-old culture of each organism, placed in the center of 100-mm petri dishes, and incubated in a Percival Intellus Environmental Controller Chamber (Perry, IA) at 25°C with a 12-h light-and-dark cycle. Colony diameter was measured with electronic calipers every day for 10 days (*M. amaranthi*) or every 2 days for 14 days (*P. amaranthicola*); *M. amaranthi* is a faster-growing organism. Petri dishes were flooded with 10 ml of sterile, deionized water and spores dislodged with a rubber spatula, following the method of Wyss et al. (35). Conidial concentrations

were measured with the aid of a Fuchs-Rosenthal hemacytometer. There were eight replicated petri dishes of each organism per herbicide concentration tested. The experiment was conducted twice for each organism.

Field trials. Two years of field trials were conducted to evaluate the effectiveness of these two organisms as biocontrol agents for common waterhemp and pigweeds in small irrigated and nonirrigated pumpkin and soybean plots. In 2008, nonirrigated pumpkin plots were located in Macomb, IL at the Western Illinois University (WIU) Agricultural Field Laboratory and irrigated pumpkin plots were located in a grower's field with center-pivot irrigation near Green Valley, IL. On 6 May and 21 May 2008, 'Libby Select' pumpkin seeds were planted at a rate of 1 seed per 2.54 cm and 1 seed per 30 cm on 76-cm row spacing at the Green Valley and WIU sites, respectively. Pumpkin seedlings were thinned to one plant every 51 to 61 cm, within the row spacing, when seedlings reached the first-true-leaf stage.

Irrigated and nonirrigated soybean plots were located in the same field at the Central Illinois Irrigated Growers Association research farm near Havana, IL. In 2008, irrigation was by center-pivot and care was taken to locate the nonirrigated plots sufficiently outside the range of the irrigation swath. On 22 May 2008, Asgrow soybean '3203RR' was planted at 395,200 seed ha⁻¹ in 76-cm rows. Plots were four rows wide. Soybean plots were not harvested due to low and nonuniform waterhemp and pigweed pressure in both areas of the field. In 2008, all field plots were a randomized complete block design with four replicated blocks.

In 2009, plots were located only at the Macomb and Havana, IL sites. The Macomb site had a natural stand of waterhemp and pigweed; however, due to weed pressure and uniformity problems, waterhemp and redroot pigweed seed were broadcast over the Havana plot area at a rate of 3.1 liters/ha on 22 May 2009. Soybean 'FS HiSoy Brand HS29R72' was planted in Havana later that day at 382,850 seeds/ha in 76-cm rows. The sprayed and harvested plot area was four rows wide by 152 cm long. In Macomb, soybean variety 'Pioneer 93Y70' was planted on 29 May 2009 at 444,600 seeds/ha drilled in 19-cm rows. At the Macomb site, each sprayed and harvested plot area was 152 cm wide by 152 cm long. In 2009, both pumpkin and soybean field plot designs were a split-block design with four replicated blocks, where herbicide treatment was the main effect and irrigation was the subunit effect. The irrigation effect was simulated by applying water to the random half of the split block in each replicated block at a rate of 1 liter per subplot, 30 min after bioherbicide and herbicide treatments were applied.

Spore suspensions of *M. amaranthi* and *P. amaranthicola* were prepared as described above, with each organism alone and as a mixture of both organisms, and amended with 10% soybean oil and 0.1% lecithin to act as an emulsifier. In 2008, the concentrations of spore suspensions for the Green Valley, Havana, and Macomb sites were *M. amaranthi* at 1.1×10^6 conidia ml⁻¹, *P. amaranthicola* at 2.7×10^6 conidia ml⁻¹, and the mixture containing *M. amaranthi* and *P. amaranthicola* at 1.1×10^6 and 2.7×10^6 conidia ml⁻¹, respectively. In 2009, the concentrations of spore suspensions for Havana and Macomb sites were *M. amaranthi* at 1.9×10^6 conidia ml⁻¹, *P. amaranthicola* at 2.2×10^6 conidia ml⁻¹, and the mixture containing *M. amaranthi* and *P. amaranthicola* at 1.9×10^6 and 2.2×10^6 conidia ml⁻¹, respectively.

Spore suspensions and herbicides were sprayed at the four- to six-leaf growth stage (2.5 to 7.6 cm) of the weeds. The treatments included spore suspensions of each fungal pathogen alone, a mixture of both pathogens, and sequential treatments with either halosulfuron-methyl (Sanda Herbicide) in pumpkin or glyphosate (Roundup Original Max Herbicide) in soybean. Halosulfuron-methyl was applied at the full labeled rate while glyphosate was applied at 18% of full rate to simulate a resistant population response and ensure that the treatment would not eliminate the weeds and inhibit fungal infection. In 2008, spore suspensions were applied at 187 liters ha⁻¹ using a handheld spray bottle and herbicide treatments were applied using a CO₂-pressurized backpack sprayer with a handheld boom having six 8002VS Teejet nozzles calibrated

to deliver the treatments at 187 liters ha⁻¹ at 33 psi. In 2009, all treatments were applied using a CO₂-pressurized backpack sprayer with handheld booms having six 8002VS Teejet nozzles calibrated to deliver the spore suspensions at 374 liters ha⁻¹ and herbicide treatments at 187 liters ha⁻¹ at 15 psi. In 2008, treatments were applied in Macomb on 11 June and in Havana and Green Valley on 12 June. In 2009, treatments were applied at Havana on 17 June and at Macomb on 25 June. HOBO H8 Pro Series data loggers (Onset Computer Corporation, Pocasset, MA) were set in each field to record temperature, relative humidity, and dew point for the first 72 h after application.

Each year, the effectiveness of bioherbicide treatments were evaluated twice at 5 to 7 and 13 to 14 days after application for their influence on disease incidence, DS, and percent weed control. Weed biomass was determined on weeds harvested at 14 days after treatment (DAT). Percent weed control was visually estimated for the entire treated area. Two random, representative 30-cm² areas of each subplot were selected to evaluate disease parameters and biomass. DS was evaluated on a scale of 0 to 5; where 0 = no visible symptoms, 1 = leaves with small necrotic flecks, 2 = small lesions on the leaves or stem, 3 = stem lesion and plant wilting, 4 = girdling stem lesion or leaf necrosis, and 5 = plant dead or girdled and falling over (modified from Smith et al.; 30). A DS index (DSI) was calculated for each plot and subplot as (mean DS × percent disease incidence)/5; where severity is rated on a 0-to-5 scale (2). Disease incidence was determined as the percentage of weeds in each 30-cm² area showing disease symptoms. A visual evaluation of percent weed control was taken for the entire 1.5-by-1.5-m treated area. In 2008, in plots having low and nonuniform waterhemp and pigweed populations, all waterhemp and pigweeds in the plot area were flagged; these plants were evaluated throughout the course of the study and then harvested 14 DAT for determining weed biomass. In plots with uniform weed populations, all waterhemp and pigweeds in two random 30-cm² areas of each subplot were harvested 14 DAT by cutting the stems at ground level and collecting all aboveground plant tissue. All harvested weed tissue was fully dried (3 days) at 82°C, and then weighed for biomass.

In this small-plot study, pumpkin yield was not possible to determine with any accuracy and, therefore, was not an objective of this research. In 2008, soybean plots were not harvested due to low and nonuniform waterhemp and pigweed populations in soybean field plots prior to bioherbicide application. In 2009, soybean yield was obtained by harvesting the center two rows of each plot at the Havana site on 20 October 2009, and by harvesting all rows in the drilled plots at the Macomb site on 4 November 2009. Plots were harvested using a research plot combine, and soybean seed was weighed and analyzed for moisture content. Soybean seed weight was standardized to 13% moisture.

Statistical analysis. The statistical design for the herbicide compatibility study was a completely randomized design with eight replicates. The experiment was conducted twice. Data were analyzed using a repeated measures analysis in PROC GLM in SAS (SAS Institute Inc., Cary, NC) for radial growth comparisons, followed by PROC REG to predict a rate response. Field data from 30-cm² plot areas were pooled prior to analysis. Spore count data and all field data were analyzed using the general linear model procedure (PROC GLM) in SAS (SAS Institute Inc.). Fisher's protected least significant difference ($P \leq 0.05$) was used to compare spore counts, DS, disease incidence, DSI, weed control, weed biomass, and yield differences among treatments and irrigation. The CORR procedure of SAS was used to compute correlation coefficients (Pearson) between weed biomass and soybean yield. The univariate procedure of SAS was used to determine that residual error terms for all variables were normally distributed; and, if necessary, variables were transformed using square-root transformation prior to analysis to equalize variance. Nontransformed means are reported with transformed P values.

Results

Herbicide compatibility study. Fungal spore production measured as spore counts after 10 days for *M. amaranthi* in culture were not significantly decreased by halosulfuron-methyl at any labeled concentration of the active ingredient but were decreased by the 2× rate (750 µg ml⁻¹). Halosulfuron-methyl prevented *P. amaranthicola* from producing fungal spores after 10 days at all rates tested (Table 1).

There was a significant effect of concentration of halosulfuron-methyl on the radial growth of each organism in culture ($P < 0.0001$). All concentrations of halosulfuron-methyl, except 250 µg ml⁻¹, significantly ($P < 0.0001$) decreased radial growth of *M. amaranthi* compared with its growth on the untreated V8 medium (Fig. 1). Differences ($P < 0.0001$) in radial growth among treatments were observed within 2 days (*M. amaranthi*) and 7 days (*P. amaranthicola*) (Figs. 1 and 2). Radial growth of *P. amaranthicola* was significantly reduced by all concentration of halosulfuron-methyl in trial 1. However, growth of the organism in trial 2 appeared reduced compared with growth in trial 1. Therefore, it is assumed that culture age may have affected general growth at all concentrations, because significant differences in radial growth were not observed between any labeled concentration even though the organism produced no spores. There were significant ($P < 0.0001$) trial and trial-date effects; therefore, trials 1 and 2 were not combined for either organism.

Field trials. Both 2008 and 2009 were exceptionally wet in Western Illinois. In 2008, the Green Valley and Havana locations received approximately 0.12 in. of rainfall within approximately 12 h after application. At these sites, data from HOBO loggers suggest

Table 1. Spore counts from petri-dish bioassay after inoculation with either *Microspora amaranthi* or *Phomopsis amaranthicola* grown on V8 agar with different concentrations of Sandea Herbicide (halosulfuron-methyl)

Halosulfuron-methyl (µg/ml) ^y	Spores per milliliter (CFU) ^w		
	<i>M. amaranthi</i> ^z	<i>P. amaranthicola</i> ^x	
		1 October	14 December
0	1,004,625 a	558,375 a	1,331,063 a
188	984,969 a	0 b	0 b
285	857,469 a	0 b	0 b
375	864,313 a	0 b	0 b
750	620,531 b	0 b	0 b
LSD (0.05)	205,924	70,468	161,607
CV	22	33	30
Mean	866,381	111,675	266,212
Prob (F)	0.0094	<0.0001	<0.0001

^w CFU data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means. Means followed by the same letter in a column are not significantly different at ($P < 0.05$) using Fisher's protected least significant difference (LSD).

^x Means are separated by date because of a significant date-treatment interaction.

^y Halosulfuron-methyl active ingredient incorporated as formulated Sandea Herbicide (75% a.i.). CV = coefficient of variation.

^z Means are presented for treatment across trials because there was no significant interaction.

that conditions were favorable for dew formation for a 1- to 2-h period prior to the rainfall. The time lag between bioherbicide application and possible leaf surface moisture was about 6 to 7 h in Green Valley and 9 to 12 h in Havana. The Macomb site, on the other hand, did not receive any rainfall within 24 h of application; however, HOBO loggers suggest that conditions were favorable for dew formation (when ambient temperature = dew point temperature) within 8 to 9 h after application.

In 2009, the Havana site received approximately 0.05 in. of rainfall within 12 h after application; in addition, HOBO loggers suggest that conditions were favorable for dew formation within 2 to 3 h after application, providing 9 to 10 h of possible leaf surface moisture prior to supplemental rainfall. The Macomb site did not receive any recorded rainfall until 48 h after application in 2009, but HOBO loggers suggest that conditions were favorable for dew formation within 3 to 5 h after application, providing possibly 7 to 8 h of leaf surface moisture (HOBO logger, data not shown).

In 2008, at the Green Valley site, air temperature within 24 h after application averaged 24°C, with a minimum of 19°C and a high of 34°C. Relative humidity averaged 74%, with a high of 94% and a low of 50% during this time period. At the Havana site, temperatures were similar to Green Valley but application occurred earlier in the evening; therefore, organisms were exposed to about 9 h of temperatures above their optimum growth range. Air temperature in both the irrigated and nonirrigated plots averaged 25°C, with a maximum of 33°C and a minimum of 19 within 24 h after application. Relative humidity ranged from 45 to 95%, with an average of 75%, during this same 24-h period. At the Macomb site, organisms were exposed to an air temperature range of 18 to 29°C, with a temperature average of 23°C for the first 24 h after application. Values for relative humidity ranged from 42 to 87% and averaged 72% during the first 24 h after application.

In 2009, air temperatures at the Havana site 24 h following application averaged 25°C and ranged from lows of 19°C followed by daytime highs of 33°C for 2 days in a row. Relative humidity at this site averaged 85%, ranging from 69 to 103% during the 24-h period following application. At the Macomb site, organisms were exposed to air temperature averaging 29°C and ranging between 23 and 37°C during the first 24 h after application. Relative humidity at this site averaged 69% and ranged from 36 to 100% within 24 h after application. Within 32 h after application, minimum air temperatures dropped to 18°C.

Disease incidence in both years, locations, and crops was significantly greater three out of five times on weeds in plots treated with the mixture of both organisms or with either glyphosate or halosulfuron-methyl followed by each organism or the mixture compared with plots treated with either organism alone (Tables 2, 3, and 4). Irrigation did not significantly impact disease incident in any year, location, or crop, and no interactions were significant; therefore, disease incident means are reported across irrigation treatments. However, irrigation did significantly impact DS ($P = 0.0002$) in 2008 at the Havana soybean location and there was a significant treatment-irrigation or -field location interaction ($P = 0.02$). Across all treatments, significantly higher DS ($P = 0.0002$) and DSI ($P = 0.0001$) scores were observed at Havana in nonirrigated soybean plots (1.8 and 35.9 for DS and DSI, respectively) compared with irrigated soybean plots (1.2 and 20.5 for DS and DSI, respectively). In most cases, DS and DSI were higher when weeds were first treated with glyphosate and then treated with either of the fungal organisms or the mixture of both organisms.

Although no consistent trend was observed, disease incidence and DS and the calculated DSI score in pumpkin and soybean (Tables 2, 3, and 4) were frequently significantly greater when the bioherbicide organisms were used in combination with either glyphosate or halosulfuron-methyl compared with the organisms alone. However,

Fig. 1. Halosulfuron-methyl dose response effects on the growth of *Microsphaeropsis amaranthi*. Radial growth of mycelium was measured on media with halosulfuron-methyl at different concentrations (0, 0.19, 0.29, 0.38, and 0.75 g/liter) compared with a control medium. Ratings for the trial were taken at 2 days after treatment. Radial growth for trial 1: $Y = -3.4998X + 11.838$, $R^2 = 0.47$; trial 2: $Y = -5.7615X + 17.302$, $R^2 = 0.62$.

Fig. 2. Halosulfuron-methyl dose response effects on the growth of *Phomopsis amaranthicola*. Radial growth of mycelium was measured on media with halosulfuron-methyl at different concentrations (0, 0.19, 0.29, 0.38, and 0.75 g/liter) compared with a control medium. Ratings for the trial were taken at 7 days after treatment. Radial growth for trial 1: $Y = -3.3608X + 8.1363$, $R^2 = 0.45$; trial 2: $Y = -3.0418X + 11.554$, $R^2 = 0.35$.

the mixture of the organisms alone frequently preformed as well as the bioherbicide and herbicide combinations. For the most part, as observed at the Green Valley site in 2008, the order of the combined treatments, whether the bioherbicide organisms were applied before or after the halosulfuron-methyl, did not significantly affect disease incidence and DS or the calculated DSI. However, in Macomb in both years, DS at 7 DAT was greater when halosulfuron-methyl was applied followed by either *M. amaranthi* or *P. amaranthicola* than when either organism was applied before halosulfuron-methyl (Table 4). However, the opposite trend was true at 14 DAT for these organisms alone and for the mixture of both organisms.

In 2008, weed control was not evaluated in the soybean plots at the Havana, IL location due to low *Amaranthus* spp. weed pres-

sure. In 2009, percent weed control in soybean plots was greatest for the glyphosate followed by *M. amaranthi* treatment at both locations. Weed control and yield were favorable when glyphosate was combined with the bioherbicide organisms, corroborating the finding of increased DS when both are combined (Table 5). In addition, the glyphosate followed by *M. amaranthi* treatment provided significantly higher weed control than glyphosate alone, except in the irrigated subplots at Havana. At the Havana location, the percentage of overall weed control was significantly ($P < 0.0001$) greater in the nonirrigation subplots (44%) compared with the irrigated (39%) and there was a significant treatment-irrigation interaction; therefore, means were separated by irrigation. However there were no significant differences or interactions between

Table 2. Percent disease incidence (DI), disease severity (DS), and disease severity index (DSI) for *Amaranthus* spp. weeds in soybean at Havana, IL after inoculation with either *Microsphaeropsis amaranthi*, *Phomopsis amaranthicola*, a mixture of both organisms, or Roundup Original MAX Herbicide (glyphosate) followed by (fb) the organisms^w

Treatment ^z	2008 ^x					2009 ^y					
	DI (%)	DS		DSI		DI (%)		DS		DSI	
		IR	Non-IR	IR	Non-IR	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
<i>M. amaranthi</i>	80 bc	2.1 a	1.3 c	15 b	26 b	78 ab	99 a	1.0 c	1.0 b	16 b	20 b
<i>P. amaranthicola</i>	72 c	0.9 ab	1.4 c	15 b	27 b	57 c	95 a	1.0 c	1.0 b	11 b	19 b
Mixture	96 a	1.1 ab	1.3 c	23 ab	24 b	65 bc	96 a	1.0 c	1.0 b	13 b	19 b
Gly fb <i>M. amaranthi</i>	92 ab	1.8 a	3.3 b	31 a	65 a	83 ab	98 a	3.9 a	1.8 a	69 a	36 a
Gly fb <i>P. amaranthicola</i>	93 ab	2.1 a	4.4 a	41 a	88 a	83 ab	92 a	2.9 b	1.5 ab	49 a	28 ab
Gly fb mixture	99 a	2.1 a	4.0 ab	41 a	78 a	87 a	93 a	3.6 ab	1.5 ab	63 a	29 ab
Glyphosate	0 d	0.0 b	0.0 d	0 c	0 c	0 d	0 b	0.0 d	0 c	0 c	0 c
Control	0 d	0.0 b	0.0 d	0 c	0 c	0 d	0 b	0.0 d	0 c	0 c	0 c
LSD (0.05)	14	1.4	0.9	18	20	18	8	0.8	0.7	16	14
CV	22	113.0	48.0	41	34	13	5	39.0	54.0	25	25
Mean	65	1.2	1.8	21	36	57	72	1.7	1.0	28	19
Prob (F)	<0.0001	<0.008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.005	<0.0001	<0.0001

^w Percent DI and DSI data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means. DSI for fungal organisms on *Amaranthus* spp. weeds was calculated by (percent incidence × mean severity)/5, where DS was rated on 0-to-5 scale: 0 = no symptoms, 1 = small necrotic flecks, 2 = discrete lesions on stem or leaves, 3 = stem lesion or plant wilting, 4 = girdling stem lesions, and 5 = plant dead or girdled and falling over. Means followed by the same letter in a column are not significantly different at ($P < 0.05$).

^x DI means for 2008 are presented for treatment across date and irrigation because there was no significant interaction. DS and DSI means for 2008 are separated by irrigation because of a significant treatment-irrigation interaction. IR = irrigated, Non-IR = not irrigated; 2008, central pivot irrigation; 2009, irrigation with hand sprayer.

^y Means are separated by date but not irrigation because of a significant date-treatment interaction; DAT = days after treatment.

^z *M. amaranthi* concentrations for 2008 and 2009 = 1.1×10^6 and 1.9×10^6 conidia ml⁻¹, respectively. *P. amaranthicola* concentrations for 2008 and 2009 = 2.7×10^6 and 2.2×10^6 conidia ml⁻¹, respectively. Mixture of *M. amaranthi* and *P. amaranthicola* for 2008 and 2009 = $1.1 \times 10^6 + 2.7 \times 10^6$ and $1.9 \times 10^6 + 2.2 \times 10^6$ conidia ml⁻¹, respectively. Glyphosate (Gly) = Roundup Original MAX Herbicide at 157 g ha⁻¹. LSD = least significant difference and CV = coefficient of variation.

Table 3. Percent disease incidence (DI), disease severity (DS), and disease severity index (DSI) for *Amaranthus* spp. weeds in soybean at Macomb, IL in 2009 after inoculation with either *Microsphaeropsis amaranthi*, *Phomopsis amaranthicola*, a mixture of both organisms, or Roundup Original MAX Herbicide (glyphosate) followed by (fb) the organisms^y

Treatment ^z	DI (%)		DS	DSI	
	7 DAT	14 DAT		7 DAT	14 DAT
<i>M. amaranthi</i>	86 b	93 c	1.0 b	17 c	19 d
<i>P. amaranthicola</i>	88 b	99 ab	1.0 b	18 c	20 cd
Mixture	99 a	98 b	1.0 b	20 c	20 cd
Glyphosate fb <i>M. amaranthi</i>	97 a	97 b	1.3 a	27 b	24 a
Glyphosate fb <i>P. amaranthicola</i>	98 a	99 ab	1.3 a	32 a	21 bc
Glyphosate fb mixture	98 a	100 a	1.2 ab	25 b	23 ab
Glyphosate	0 c	0 d	0.0 c	0 d	0 e
Control	0 c	0 d	0.0 c	0 d	0 e
LSD (0.05)	4	2	0.2	5	2
CV	3	1	31.0	10	7
Mean	71	73	0.9	17	16
Prob (F)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^y Percent DI and DSI data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means. Means are separated by date because of a significant date-treatment interaction; DAT = days after treatment. DS means are presented for treatment across date and irrigation because the was no significant interaction. Means followed by the same letter in a column are not significantly different at ($P < 0.05$). DSI of fungal organisms on *Amaranthus* spp. weeds calculated by (percent incidence × mean severity)/5, where DS was rated on 0-to-5 scale: 0 = no symptoms, 1 = small necrotic flecks, 2 = discrete lesions on stem or leaves, 3 = stem lesion or plant wilting, 4 = girdling stem lesions, and 5 = plant dead or girdled and falling over.

^z *M. amaranthi* concentration = 1.9×10^6 conidia ml⁻¹, *P. amaranthicola* concentration = 2.2×10^6 conidia ml⁻¹, mixture of *M. amaranthi* and *P. amaranthicola* concentration = $1.9 \times 10^6 + 2.2 \times 10^6$ conidia ml⁻¹, respectively, and Glyphosate = Roundup Original MAX Herbicide at 157 g ha⁻¹. LSD = least significant difference and CV = coefficient of variation.

evaluation dates. Irrigation had no significant impact on weed control in soybean plots at the Macomb location; however, the percentage of weeds controlled significantly ($P < 0.0001$) increased between the 7- and 14-DAT evaluation dates (16 and 21%, respectively). A significant interaction existed between treatment and evaluation date; therefore, means were separated by date (Table 5).

In pumpkin plots, irrigation had no significant impact on weed control at either location. In 2008, weed control was not evaluated in the pumpkin plots at the Green Valley, IL location due to low *Amaranthus* spp. weed pressure. At the Macomb site, percent weed control was significantly different at both rating dates and there was a significant treatment–date interaction; therefore weed control

Table 4. Percent disease incidence (DI), disease severity (DS), and disease severity index (DSI) for *Amaranthus* spp. weeds in pumpkin at Green Valley (under center-pivot irrigation) and Macomb, IL (nonirrigated) after inoculation with either *Microsphaeropsis amaranthi*, *Phomopsis amaranthicola*, a mixture of both organisms, or Sandea Herbicide (halosulfuron-methyl) before or followed by (fb) the organisms^w

Treatment ^z	Green Valley, IL ^y			Macomb, IL ^x							
				2008 ^x				2009			
				DS		DI (%)		DS		DSI	
	DI (%)	DS	DSI	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
<i>M. amaranthi</i>	93 a	2.1 ab	39 abc	0.6 de	0.5 ab	91 b	100 a	1.0 c	1.0 b	18 e	20 c
<i>P. amaranthicola</i>	89 ab	0.9 c	17 d	0.5 e	0.0 c	92 b	100 a	1.0 c	1.0 b	18 e	20 c
Mixture	89 ab	1.3 bc	24 cd	0.5 e	0.5 ab	99 a	100 a	1.0 c	1.0 b	20 de	20 c
Halo fb <i>M. amaranthi</i>	100 a	2.3 ab	46 a	2.4 a	0.6 ab	99 a	100 a	1.8 a	1.3 ab	35 ab	25 abc
Halo fb <i>P. amaranthicola</i>	87 ab	1.5 bc	27 cd	2.1 ab	0.5 a	97 a	100 a	1.6 ab	1.6 a	31 bc	31 a
Halo fb mixture	100 a	2.1 ab	41 ab	1.5 bc	0.4 ab	98 a	100 a	1.2 c	1.6 a	23 d	31 a
<i>M. amaranthi</i> fb halo	81 b	2.0 ab	38 abc	1.1 cd	0.4abc	100 a	100 a	1.4 b	1.3 ab	29 c	25 abc
<i>P. amaranthicola</i> fb halo	95 a	1.4 bc	26 bcd	1.5 bc	0.4 ab	92 b	100 a	1.1 c	1.0 b	20 e	20 c
Mixture fb halo	100 a	1.9 ab	38 abc	1.3 c	0.4 ab	100 a	100 a	1.8 a	1.2 b	35 a	24 bc
<i>M. amaranthi</i> halo TM	...	...	...	...	...	99 a	100 a	1.7 a	1.3 ab	33 abc	26 ab
Halosulfuron	0 c	0.0 d	0 e	0.0 f	0.1 bc	0 c	0 b	0.0 d	0.0 c	0 f	0 d
Control	0 c	0.0 d	0 e	0.0 f	0.0 c	0 c	0 b	0.0 d	0.0 c	0 f	0 d
LSD (0.05)	14	0.7	15	0.7	0.5	3	0	0.2	0.3	5	7
CV	15	52.0	30	48.0	101	2	0	20.0	33.0	10	13
Mean	75	1.4	27	1.0	0.3	81	83.3	1.1	1.0	22	20
Prob (<i>F</i>)	<0.0001	<0.0001	<0.0001	<0.0001	0.0096	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^w Weed percent DI and DSI data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means. Means followed by the same letter in a column are not significantly different at ($P < 0.05$). DSI of fungal organisms on *Amaranthus* spp. weeds calculated by (percent incidence $\times$ mean severity)/5, where DS was rated on 0-to-5 scale: 0 = no symptoms, 1 = small necrotic flecks, 2 = discrete lesions on stem or leaves, 3 = stem lesion or plant wilting, 4 = girdling stem lesions, and 5 = plant dead or girdled and falling over.

^x Means are separated by date because of a significant date–treatment interaction; DAT = days after treatment.

^y Means are presented for treatment across date and irrigation because there was no significant interaction.

^z *M. amaranthi* concentration in 2008 and 2009 = 1.1×10^6 and 1.9×10^6 conidia ml⁻¹, respectively. *P. amaranthicola* concentration in 2008 and 2009 = 2.7×10^6 and 2.2×10^6 conidia ml⁻¹, respectively. Mixture of *M. amaranthi* and *P. amaranthicola* in 2008 and 2009 = $1.1 \times 10^6 + 2.7 \times 10^6$ and $1.9 \times 10^6 + 2.2 \times 10^6$ conidia ml⁻¹, respectively. Halosulfuron-methyl (Halo) = Sandea Herbicide at 70 g ha⁻¹. TM = tank mix, LSD = least significant difference, and CV = coefficient of variation.

Table 5. Soybean yield (kg/ha), percent *Amaranthus* spp. weed control (WC) and weed weight in soybean at Havana and Macomb, IL after inoculation with either *Microsphaeropsis amaranthi*, *Phomopsis amaranthicola*, a mixture of both organisms, or Roundup PowerMAX Herbicide (glyphosate) followed by (fb) the organisms^v

Treatment ^z	Havana, IL ^w						Macomb, IL			
	2008		2009				2009			
	Wt (g)		WC (%)		Wt (g) ^y	Yield	WC (%) ^x		Wt (g) ^y	Yield
	IR	Non-IR	IR	Non-IR			7 DAT	14 DAT		
<i>M. amaranthi</i>	0.141 c	0.534 bc	0 d	0 d	0.475 bc	3,147.8 a	12 e	12 d	0.557 b	3,909.4 c
<i>P. amaranthicola</i>	0.399 ab	0.411 bc	0 d	0 d	0.650 b	2,838.8 a	0 f	0 e	0.452 bc	4,133.5 c
Mixture	0.327 ab	0.373 cd	0 d	0 d	0.507 bc	2,714.5 a	0 f	0 e	0.459 bc	4,282.9 c
Gly fb <i>M. amaranthi</i>	0.282 abc	0.424 cd	83 a	95 a	0.275 cd	3,456.9 a	38 a	53 a	0.315 c	5,503.0 ab
Gly fb <i>P. amaranthicola</i>	0.363 abc	0.113 d	74 c	78 c	0.285 cd	3,119.2 a	18 d	27 c	0.424 bc	5,801.8 a
Gly fb mixture	0.166 bc	0.118 cd	76 bc	88 b	0.112 d	2,488.3 a	31 b	38 b	0.312 c	5,602.6 a
Glyphosate	0.443 a	1.223 b	79 ab	89 b	0.143 d	3,090.5 a	26 c	41 b	0.330 c	5,478.1 ab
Control	0.555 a	2.544 a	0 d	0 d	3.285 a	2,517.0 a	0 f	0 e	1.349 a	4,980.1 b
LSD (0.05)	0.3	1.0	5	4	0.7	NS	5	4	0.293	531.7
CV	29.0	40.0	5	4	30.0	20	14	8	19.0	10.0
Mean	0.34	0.75	39	44	0.72	2,926	16	21	0.525	4,961.4
Prob (<i>F</i>)	0.04	<0.0001	<0.0001	<0.0001	<0.0001	0.14	<0.0001	<0.0001	<0.0001	<0.0001

^v Means followed by the same letter in a column are not significantly different at ($P < 0.05$); NS = not significant. DAT = days after treatment. Weed weight (Wt) and percent WC data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means.

^w Means are separated by irrigation because of a significant treatment–irrigation interaction. IR = irrigated and Non-IR = not irrigated. 2008, central pivot irrigation; 2009, irrigation with hand sprayer.

^x Means are separated by date because of a significant date–treatment interaction.

^y Weed weight at 14 DAT.

^z *M. amaranthi* concentration in 2008 and 2009 = 1.1×10^6 and 1.9×10^6 conidia ml⁻¹, respectively. *P. amaranthicola* concentration in 2008 and 2009 = 2.7×10^6 and 2.2×10^6 conidia ml⁻¹, respectively. Mixture of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* in 2008 and 2009 = $1.1 \times 10^6 + 2.7 \times 10^6$ and $1.9 \times 10^6 + 2.2 \times 10^6$ conidia ml⁻¹, respectively. Glyphosate (Gly) = Roundup Original MAX Herbicide at 157 g ha⁻¹. LSD = least significant difference and CV = coefficient of variation.

means are presented by date (Table 6). General weed control significantly decreased ($P < 0.0001$) in 2008 between 7 and 14 DAT (30 and 12%, respectively) and, in 2009, weed control significantly ($P < 0.0001$) increased (3 and 12%, respectively). In 2008, all halosulfuron-methyl and bioherbicide treatments reduced weed infestations relative to the untreated control, and most sequential treatments of the bioherbicide organisms with halosulfuron-methyl provided weed control similar to halosulfuron-methyl alone (Table 6). However, in 2009, due to high weed infestations, performance of all bioherbicide and herbicide treatments in pumpkin plots was lower than that observed in 2008. In addition, weed control in the pumpkin plots in 2009 by halosulfuron-methyl was zero. Weed control was significantly increased by all sequential applications of the bioherbicide treatments with halosulfuron-methyl at 7 DAT, and most showed a significant increase in weed control at 14 DAT (Table 6). Tank mixing *M. amaranthi* with Sandea provided weed control similar to the sequential treatment of *M. amaranthi* followed by halosulfuron-methyl.

Reductions in weed dry weight were significant between soybean plots in the irrigated section of the field compared with plots in the nonirrigated area at the Havana site in 2008, and an interaction existed between irrigation, field location, and treatment. Therefore, weed biomass values are separated by irrigation for the Havana location in 2008. Overall, dry weight of weeds harvested from Havana soybean plots in the irrigated section were significantly smaller ($P = 0.02$) than weeds harvested from plots in the nonirrigated section of the field. In the irrigated plots, weeds treated with glyphosate followed by the mixture of both organisms or with *M. amaranthi* alone had significant reductions in biomass compared with plots treated with glyphosate alone or the untreated control. In the nonirrigated plots, all bioherbicide treatments significantly reduced weed biomass compared with the untreated control. In addition, the mixture alone and treatments with glyphosate followed by either organism or the mixture significantly reduced weed biomass compared with glyphosate alone (Table 5). In 2009, irrigation did not significantly impact weed biomass at either the Havana or the Macomb site. All the bioherbicide and herbicide treatments significantly reduced weed biomass compared with the untreated control at 14 DAT at both locations. However,

the Macomb site did not show significantly different reductions in weed biomass among bioherbicide and herbicide treatments whereas, at the Havana location, glyphosate or glyphosate followed by the bioherbicide organisms significantly reduced weed biomass compared with the treatments with the bioherbicide organisms alone. The greatest reduction in weed weight was observed when glyphosate was followed by the mixture of both organisms at both sites (Table 5).

Reductions in *Amaranthus* spp. weed biomass in pumpkin plots were significant for all treatments of the bioherbicide organisms, halosulfuron-methyl alone, and all sequential treatments of the bioherbicide organisms with halosulfuron-methyl compared with the untreated control (Table 6). The Green Valley site in 2008 showed the most dramatic reductions in weed biomass. Biomass of weeds from plots treated with any bioherbicide or combination treatment was significantly reduced compared with plots treated with halosulfuron-methyl alone. Whether the bioherbicide organisms were applied before or after halosulfuron-methyl did not appear to influence weed biomass in any year or location.

Yield was taken in soybean plots at both locations in 2009 (Table 5). At the Macomb site, soybean yield from plots treated with glyphosate followed by *P. amaranthicola* or by the mixture of both organisms had significantly higher yield than the untreated control plots or plots treated with the organisms alone. At Macomb, a significant ($P = 0.01$) negative correlation ($r = -0.30$) existed between weed weight and soybean yield. Soybean yield at the Havana site did not show significant treatment differences or correlations with weed weight but yield obtained in the plot treated with glyphosate followed by *M. amaranthi* was the highest. The large number of common lambsquarters (*Chenopodium album*), black nightshade (*Solanum ptycanthum*), and ivyleaf morningglory (*Ipomoea hederacea*) at the Havana site was probably responsible, in part, for lower yields and lack of treatment differences.

Discussion

Although performances of the bioherbicides were variable and, in some cases, marginal, this research demonstrated the potential for the integration of these bioherbicides into crop production systems using glyphosate and into vegetable production systems using

Table 6. Percent *Amaranthus* spp. weed control (WC) and weed weight in pumpkin at Green Valley and Macomb, IL after inoculation with either *Microsporaopsis amaranthi*, *Phomopsis amaranthicola*, a mixture of both organisms, or Sandea Herbicide (halosulfuron-methyl) before or followed by (fb) organisms^y

Treatment ^z	Green Valley 2008		Macomb 2008		Macomb 2009		
	Weed wt (g)	WC (%)	Weed wt (g)	WC (%)	Weed wt (g)	WC (%)	Weed wt (g)
	14 DAT	7 DAT	14 DAT	14 DAT	7 DAT	14 DAT	14 DAT
<i>M. amaranthi</i>	1.816 cd	19 de	5 bc	0.159 b	0 c	0 c	0.318 b
<i>P. amaranthicola</i>	1.059 cd	19 cde	3 c	0.225 b	0 c	0 c	0.275 bcd
Mixture	2.860 c	10 e	19 ab	0.198 b	0 c	0 c	0.261 bcd
Halo fb <i>M. amaranthi</i>	0.996 d	51 a	14 ab	0.229 b	8 b	18 b	0.273 bcd
Halo fb <i>P. amaranthicola</i>	2.108 cd	35 abc	15 ab	0.191 b	8 b	24 a	0.213 de
Halo fb mixture	1.380 cd	40 ab	14 ab	0.137 b	8 b	24 a	0.296 bc
<i>M. amaranthi</i> fb halo	1.041 d	28 bcd	18 a	0.166 b	10 a	25 a	0.191 e
<i>P. amaranthicola</i> fb halo	0.860 d	35 ab	18 a	0.150 b	0 c	0 c	0.274 bcd
Mixture fb halo	0.960 d	39 ab	13 ab	0.145 b	8 b	25 a	0.253 bcde
<i>M. amaranthi</i> halo TM	...	...	...	...	0 c	18 b	0.233 cde
Halosulfuron	16.751 b	53 a	18 a	0.262 b	0 c	16 b	0.210 de
Control	33.346 a	0 f	0 c	0.510 a	0 c	0 c	0.894 a
LSD (0.05)	4.9	16	13	0.17	2	2	0.09
CV	30.0	27	45	26.0	28	6	12.0
Mean	6.0	30	12	0.22	3	12.4	0.31
Prob (<i>F</i>)	<0.0001	<0.0001	0.0011	0.0698	<0.0001	<0.0001	<0.0001

^y Weed weight and percent WC data were square-root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means. Means are presented for treatment across date and irrigation because there was no significant interaction; DAT = days after treatment. Means are separated by date because of a significant date-treatment interaction. Means followed by the same letter in a column are not significantly different at ($P < 0.05$).

^z *M. amaranthi* concentration in 2008 and 2009 = 1.1×10^6 and 1.9×10^6 conidia ml⁻¹, respectively. *P. amaranthicola* concentration in 2008 and 2009 = 2.7×10^6 and 2.2×10^6 conidia ml⁻¹, respectively. Mixture of *M. amaranthi* and *P. amaranthicola* in 2008 and 2009 = $1.1 \times 10^6 + 2.7 \times 10^6$ and $1.9 \times 10^6 + 2.2 \times 10^6$ conidia ml⁻¹, respectively. Halosulfuron-methyl (Halo) = Sandea Herbicide at 70 g ha⁻¹. TM = tank mix, LSD = least significant difference, and CV = coefficient of variation.

the ALS-inhibitor herbicide active ingredient halosulfuron-methyl. These results are particularly significant because of the high frequency of glyphosate- and ALS-herbicide-resistant pigweed and waterhemp biotypes in the United States (10,18).

Previous work by Wyss et al. (35) and Smith and Hallett (32) illustrated that many herbicides reduce the spore production of these organisms but this is the first time halosulfuron-methyl has been evaluated as a potential tank mix for either of these organisms. Halosulfuron-methyl appears to reduce growth and stop spore production of *P. amaranthicola*. Although both radial growth trials with *P. amaranthicola* showed mycelia growth in culture, spore production was zero for all tested concentrations of halosulfuron-methyl. And even though Roskopf et al. (20) illustrated high mortality of *Amaranthus* spp. after inoculation with mycelium alone, mixing *P. amaranthicola* with halosulfuron-methyl would not be recommended at this time. However, radial growth of *M. amaranthi* mycelium was not reduced at the lowest two concentrations of halosulfuron-methyl and spore production of *M. amaranthi* was similar to the control for all but the 2× rate. Tank mixing would provide a distinct benefit for the use of *M. amaranthi* as a bioherbicide. However, more work is needed to provide better efficacy with this method.

Both *M. amaranthi* and *P. amaranthicola* have been shown to need at least an 8- to 12-h dew period (13,19,30,34). In this study, irrigation was evaluated in order to increase leaf surface moisture and help ensure a sufficient dew period for fungal infection under field conditions. However, rainfall in both years and field locations was exceptionally above average and commercial irrigation systems, being out of the control of researchers, were not used after application. However, in nonirrigated and irrigated plots, the incidence of disease did not always equate to higher DS. HOBO data loggers (*data not shown*) recorded field conditions at the Green Valley and Havana sites as having rainfall within 12 h after application plus conditions for leaf surface moisture for greater periods of time compared with conditions recorded at the Macomb site in 2008. Rainfall following application could account for the fact that irrigation did not have much impact on results in either year. It is also possible that the simulated irrigation method used in the split-block design in 2009 may have been applied too soon after spraying the spore suspension, thus washing some spores from the surface of the leaf and lowering pathogen infection. In any case, weed control and DS at the Macomb site were generally less than that observed at the Havana site, while the Green Valley site had the greatest reduction in weed biomass. Although differences might be explained by center-pivot irrigation at the Havana and Green Valley sites, due to overly wet seasons during the 2-year duration of this study, irrigation systems were not turned on following bioherbicide application; therefore, all sites were similar with respect to irrigation. Environmental differences after application and an extremely dense population of waterhemp and pigweed at the Macomb site may be more responsible for observed differences. Roskopf (20) also reported variable results in field trials for *P. amaranthicola* between years and among *Amaranthus* spp. resulting, in part, from differences in relative humidity levels and rainfall.

In addition to leaf moisture, these organisms need optimum air temperatures for germination and infection. Mintz et al. (13) and Roskopf et al. (22) found that *M. amaranthi* and *P. amaranthicola*, respectively, can cause weed mortality at temperatures ranging from 20 to 35°C, with optimum temperatures around 28 to 30°C. Disease can occur at 20°C but was not reported at levels high enough to cause plant mortality (22). Smith et al. (30) reported temperature optima of 18 to 24°C for *M. amaranthi*. These temperature optima could explain the inconsistencies in DS at the field level and the inconsistencies seen over the 2 years of this research, where a wide range of air temperatures occurred after application.

Research plots were not selected with a known population of weeds resistant to either glyphosate or halosulfuron-methyl; therefore, it is reasonable to presume that the bioherbicide treatments might obtain better weed control compared with glyphosate and

halosulfuron-methyl if evaluated on known resistant weed populations. However, in this research, sublethal levels of glyphosate in combination with these organisms frequently exhibited better weed control compared with glyphosate or the organisms alone. In most instances, the bioherbicide and glyphosate combinations reduced weed biomass to an extent similar to results observed by Singh et al. (25–28) and Smith (29). Unfortunately, this effect with a labeled rate of halosulfuron-methyl was not as clear. In Macomb in 2009, weed control in the pumpkin plots by halosulfuron-methyl alone was zero and, even with low DS and DSI, weed control by halosulfuron-methyl was significantly improved by all sequential applications of the bioherbicide treatments at 7 DAT, with most showing an increase in weed control by 14 DAT. Weed control and biomass reduction values reflect the potential of these organisms to outperform either herbicide on resistant weed populations.

Differences in overall yield potential between the Havana and Macomb sites in 2009 result, in part, from the Havana site being planted in 30-in. rows and the Macomb site being drilled. The Havana site is primarily a sandy soil under irrigation. However, in 2009, the wet growing season did not warrant supplemental irrigation. Differences in weed control and biomass reduction may be explained more readily by environmental differences influencing the degree of pathogen infection in the hours following application. Both sites had conditions favoring some dew formation to help provide good leaf surface moisture but at the Macomb site air temperature was higher than ideal. Both sites had moderate disease incidence, meaning many weeds displayed foliar symptoms; however, the level of DS and weed control was greater at the Havana site compared with Macomb. Levels of disease may be higher if there is no lag time between application and dew period, where the spores may be less likely to desiccate on the leaf surface.

The results of this research illustrate the potential of these bioherbicide organisms to be integrated into production systems with other weed management technologies. Continued research on these and other fungal pathogens can benefit and impact weed control practices worldwide by providing needed information and knowledge about the use and application of bioherbicides.

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