Phomopsis amaranthicola and *Microsphaeropsis amaranthi* Symptoms on *Amaranthus* spp. Under South Texas Conditions

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ABSTRACT

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Temperature, humidity, weed species and age, and inducible responses in the host are factors that could limit the efficacy of fungal bioherbicides. The influences of these factors on the efficacy of the fungal bioherbicides *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* against Palmer amaranth (*Amaranthus palmeri*), smooth pigweed (*Amaranthus hybridus*), and redroot pigweed (*Amaranthus retroflexus*) were investigated in greenhouse and field studies under south Texas conditions. Despite plants being given an initial dew period, the bioherbicides, applied individually or in combination, did not cause mortality on any pigweed species in greenhouse or field environments. In greenhouse experiments, fewer than 5% of the leaves in six- to eight-leaf *A. palmeri* plants developed necrotic lesions within 2 weeks after bioherbicide treatment and only 8% or fewer of the plants developed stem lesions. Disease incidence was significantly higher in *A. hybridus* and *A. retroflexus*, with as much as 94% of leaves developing necrosis and 95% of the plants having stem lesions. New leaf production was reduced by bioherbicide treatment in *A. hybridus*. Combined-pathogen inoculation caused leaf and stem lesions on mature (13 to 36 leaves per plant) *A. hybridus* and *A. retroflexus*. Summer and fall field inoculations with *M. amaranthi* on *A. hybridus* and *A. palmeri* produced disease incidence levels similar to or higher than those in greenhouse tests. Infection of *A. palmeri* by *P. amaranthicola* increased the peroxidase activity level nearly twofold compared with the controls. Neither pathogen influenced leaf free amino acid content. The high temperatures and low humidity of south Texas and interspecific variation in resistance, possibly linked to peroxidase induction, limited the efficacy of these bioherbicides.

Additional keywords: biological control, cotton weeds, Lower Rio Grande Valley of Texas

Native fungal plant pathogens have shown promise as bioherbicides when applied inundatively against weeds in crops (2,10,11), but ambient temperature and humidity conditions that favor fungal infection (13,30,38,44,46) might not exist in all regions in which the targeted weeds require control. In addition, bioherbicide disease development may vary according to weed age (19,31) or species within the host range (29,34), limiting efficacy in mixed weed infestations (15). Adjustments in inoculum dose (13,15, 23,31,33), formulation (3,7,12,19,35,37, 40,49,50), humidity (29), and the seasonal or diurnal timing of inoculations, as well as application of multiple pathogens (9,29), can all increase bioherbicide efficacy, but may not be practical in all regions and crops.

Pigweeds (*Amaranthus* spp.) are agronomically deleterious in the United States and Europe (8) and can reduce yields of cotton and other crops by competition (14,36) exacerbated by rapid growth and copious seed production (18,39). Nine *Amaranthus* species occur in the Lower Rio Grande Valley of south Texas, (32), including Palmer amaranth (*Amaranthus palmeri* S. Wats.), redroot pigweed, (*Amaranthus retroflexus* L.), and smooth pigweed (*Amaranthus hybridus* L.). Palmer amaranth is the most damaging species regionally and grows more rapidly than other *Amaranthus* spp. (39). Although synthetic herbicides are often effective against *A. palmeri* (6,14), resistance exists to dinitroanilines, acetolactate synthase (ALS) inhibitors, photosystem II inhibitors, and glyphosate (17) and is becoming increasingly prevalent in *A. retroflexus*, *A. hybridus*, and other pigweeds (20,22), creating a need for alternative control approaches (5,8,11).

Two necrotrophic fungi, *Phomopsis amaranthicola* Roskopf, Charudattan, Shabana, & Benny and *Microsphaeropsis amaranthi* Heiny & Mintz (Ell. and Barth.), target *Amaranthus* spp. (23,24,29, 33,34,44). *P. amaranthicola* has been patented for *Amaranthus* control (U.S. Patent

Nos. 5510316 and 5393728). In greenhouse and field tests in Florida and Illinois, 90 to 100% of *A. palmeri*, *A. hybridus*, and *A. retroflexus* seedlings developed leaf and stem lesions and as much as 83% of *A. palmeri* and 100% of *A. hybridus* and *A. retroflexus* were killed within 2 to 8 weeks (29,33,34). Formulation of *P. amaranthicola* with surfactants, humectants, and other components increased efficacy (25,29,33). Symptoms and mortality differed among pigweed species (34), between greenhouse or field environments (29,44), across field trials with varying ambient humidity (33), and declined in seedlings having more than six leaves (24,35). Mortality and dry weight reductions occurred at moderate doses ($\leq 1 \times 10^6$ conidia per ml) (24,35,44) when a dew period of 12 h or more was provided after inoculation, with temperatures between 20 and 30°C (30,35).

No information is available on the efficacy of these bioherbicidal fungi in south Texas, where environmental conditions differ from regions where *P. amaranthicola* and *M. amaranthi* had been previously evaluated. Average daily maximum and mean temperatures between April and October in Weslaco, TX are 2.4°C higher than in Gainesville, FL and 8.6°C higher than in Urbana, IL, and total rainfall in Weslaco during these months is only 59 and 69% that of these two prior testing locations, respectively (28). This study evaluated symptom development in *A. palmeri*, *A. hybridus*, and *A. retroflexus* infected with *P. amaranthicola* and *M. amaranthi* under the relatively hot, dry conditions of south Texas.

Among the biochemical defenses induced in plants following infection, peroxidases can strengthen cell walls, are involved in signaling in response to pathogen infection (47), and may reduce bioherbicide efficacy when they are induced in weeds (1), but no information about the peroxidase activity level is available for pigweed bioherbicides. Changes in nutritive resources such as protein and free amino acids (FAAs) could influence pathogen growth (16), moderate plant stress (42), and alter the suitability of pigweeds for insect herbivores (26,41,42). The effects of pathogen inoculation on these defense and stress indicators in pigweeds were therefore also examined in this study.

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MATERIALS AND METHODS

Temperature and relative humidity measurement. Greenhouse experiments were conducted at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX. Temperature and relative humidity (RH) data were obtained hourly, 24 hours a day, with an HOBO H8 meter (Onset Computer Co., Bourne, MA). Field experiments were performed at the USDA-ARS Rio Delta Research Farm, Monte Alto, TX (26°25'N, 97°57'W) in a Willacy fine sandy loam (fine-loamy, mixed hyperthermic Udic Argiustolls). Temperature and RH data were obtained hourly, 24 hours a day, with a Vaisala Model HMP35AC (Wescor Inc., Logan, UT). In both greenhouse to field and field season comparisons, daily natural dew periods were determined by summing the hours per day during which RH was $\geq 95\%$. To compare greenhouse and field conditions, readings were taken during a 78-day period from 3 September to 19 November 2005. To compare summer and fall field conditions, data were collected from 1 August to 30 August and from 16 November to 15 December in 2003 and 2004 and were combined between years.

Humidity data were arcsine square root transformed. Hourly temperature and RH collected from 3 September to 19 November 2005 were compared between greenhouse and field environments with repeated-measures analysis of variance (ANOVA) using SAS PROC MIXED Version 9.1 (SAS Institute, Cary, NC). Maximum daily temperatures were compared using *t*-tests (PROC TTEST) with Satterthwaite approximations. Average daily and maximum temperatures and average humidity were also compared in a subset of the data (from 3 to 30 September 2005) that reflected summer conditions typical of most greenhouse experiments. Average greenhouse and field temperature and humidity between 2000 and 0900 h were also compared using *t*-tests. This 13-h period was selected on the basis of past studies (35,44) demonstrating that an initial dew period of 12 h or more was sufficient to produce symptoms, but that high nighttime ambient humidity and moderate temperatures after the first night contributed to bioherbicide efficacy (29,33). Similar *t*-tests were used to compare daily average temperature and RH, maximum temperature, average temperature and RH between 2000 and 0900 h, and the duration of natural dew periods between the summer and fall field environments.

Plant propagation. Seeds of *A. palmeri* (from one accession collected in Hidalgo County, TX) and *A. hybridus*, *A. retroflexus*, and *A. lividus* (obtained from R. Charudattan, Department of Plant Pathology, University of Florida, Gainesville) were sown in 8-cm-diameter pots (10 seeds per pot) in peat-based soil (Sunshine Mix No. 1; SunGro Horticulture, Bellevue,

WA). Seedlings were thinned to one per pot at 10 days old. Plants were maintained in a greenhouse under simulated summer conditions (temperature mean $\pm$ SE: $29.3 \pm 0.1^\circ\text{C}$; RH mean $\pm$ SE: $73.2 \pm 0.5\%$). Natural lighting was supplemented by 400W Master HPI-T Plus lamps (Philips, New York, NY) providing $490 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ daytime irradiance, 16:8 light/dark. Each plant was fertilized with 100 ml of a 15.8 g/liter solution of 17-17-17 N-P-K fertilizer (Scotts-Sierra, Marysville, OH).

Fungal propagation and inoculations.

P. amaranthicola (ATCC isolate No. 74226) and *M. amaranthi* (CAB IMI isolate No. 327754) were obtained from R. Charudattan under USDA-APHIS-PPQ permits 55737 and 62145, respectively, and were maintained at 27°C on a medium containing 200 ml of tomato juice, 3 g CaCO_3 and 15 g of agar L^{-1} . Two-week-old cultures were used for preparing inoculum suspensions. In the 2003 and 2004 experiments, conidia were suspended in water containing 0.5% (v/v) polyoxyethylene sorbitan monolaurate surfactant (Tween 80; Sigma, St., Louis, MO). In the 2005 experiments, conidia were suspended in water containing 0.003% (v/v) D-limonene and nonylphenol ethoxylate surfactant (IVOD; Brewer International, Vero Beach, FL), 0.05% (w/v) algal polysaccharide humectant (Kelgin LV; CP Kelco, San Diego, CA), and 0.2% (v/v) Tween 80. Formulation ingredients were added during 2005 in an effort to increase symptoms and efficacy on the basis of work with other bioherbicides (2,3,7,45,50). The limonene-based surfactant and the algal polysaccharide were both known to be compatible with *P. amaranthicola* germination (49). Most prior studies of both pigweed bioherbicides had used a polysaccharide humectant (29,34,35). Conidial suspensions were filtered through two layers of cheesecloth and adjusted to 2×10^6 conidia ml^{-1} with a hemacytometer (Bright-Line; Reichert, Buffalo, NY). For combined-pathogen inoculations, suspensions were adjusted prior to mixing to yield a final concentration of 2×10^6 conidia ml^{-1} per pathogen. Fungal inoculum and controls (formulation liquid without conidia) were applied to entire plants (3 ml per plant) with a 150-ml cosmetic pump sprayer (2003 and 2004 greenhouse and field studies) or a hand-held pressurized (480 kPa) 200-ml air sprayer (2005 greenhouse studies) (Ace Paint Pak 1065556; Ace Hardware, Oak Brook, IL). Plants were inoculated between 1830 and 2030 h and kept under plastic tents that were misted with water and then sealed for 13 h after inoculation to enhance leaf wetness. This artificial dew period was provided to allow comparisons with prior studies of bioherbicides against pigweeds (12,19,29,34,35,44) and other weeds (9,13,46) that used similar or longer dew periods.

Comparison of pigweed species. The susceptibility of the three pigweed species to the bioherbicial fungi was compared in a series of eight greenhouse experiments, with some experiments involving only some of the three pigweed species and four fungal treatments (control, two single-pathogen treatments, and one combined-pathogen treatment). Across seven of these experiments, 40 young *A. palmeri* and 51 *A. hybridus* seedlings per treatment were treated with suspensions of single pathogens or with formulation controls. In one of the eight experiments, suspensions containing both fungi were applied to a total of 13 plants of each pigweed species. In three of the eight experiments, 31 *A. retroflexus* plants were inoculated with *P. amaranthicola* and 21 plants with *M. amaranthi*; no combined-pathogen inoculations of *A. retroflexus* were performed. Fungal suspensions were applied 18 days after seedling emergence. *A. palmeri* plants were 9.6 ± 0.5 cm tall with 8 ± 1 leaves, *A. hybridus* plants were 4.9 ± 0.2 cm tall with 6 ± 0.3 leaves, and *A. retroflexus* plants were 8.0 ± 0.9 cm tall with 7 ± 1 leaves. Inoculated plants were arranged randomly within treatment groups on one greenhouse bench. The percentage of leaves on each plant with at least one necrotic lesion and the percentage of plants of each treatment that had at least one stem lesion were determined 2 weeks after inoculation. Mortality, number of new leaves (net leaf gain), and height gain were determined 3 weeks after inoculation.

Prior to statistical analysis, percent leaf necrosis data were arcsine square root transformed and leaf and height gain data were log transformed. Because leaf necrosis data were not normally distributed, leaf necrosis data were ranked across all species, treatments, and experiments for analysis. The effects of pigweed species and fungal treatment on ranked leaf necrosis, net leaf gain, and height increase were examined with ANOVA using SAS PROC MIXED with species, treatment and their interaction as fixed effects, and experiment as a random effect, followed by separate ANOVA analyses for each pigweed species to compare pathogen treatments using least-square mean comparisons with Tukey adjustment to *P* values. This procedure did not provide an estimate of the significance of the random experiment effect. The ranges of means from individual experiments for each pathogen treatment and pigweed species are presented in the text to estimate experimental variability. Counts of plants with and without stem lesions were analyzed with asymptotic maximum-likelihood χ^2 -square tests to determine overall pigweed species and treatment effects, followed by separate analyses of treatment effects in each species. Back-transformed means of transformed necrosis data with lower and upper 95% confidence intervals, percentages of

plants with stem lesions, and means $\pm$ SE's of untransformed leaf gain are presented.

Effect of plant age. In one greenhouse experiment, pigweed plants were inoculated with a combined-pathogen suspension when the plants were 35 days old and flowering. *A. palmeri* plants were 51.8 ± 7.6 cm tall with 47.9 ± 7.5 leaves, *A. hybridus* plants were 32.1 ± 8.7 cm tall with 36.3 ± 1.6 leaves, and *A. retroflexus* plants were 22.7 ± 11.9 cm tall with 12.8 ± 1.3 leaves. Twelve to fourteen plants were treated per treatment per species. Three separate experiments were conducted in which 18-day-old seedlings (14 to 15 plants per pigweed species per treatment) were treated with the combined-pathogen suspension or formulation control. Leaf necrosis and stem lesions were assessed 2 weeks after inoculation. Final leaf counts and heights were measured 3 weeks after inoculation. Ranked leaf necrosis and log-transformed leaf and height data were analyzed separately for each plant age with ANOVA (with a random experiment effect for young plants) to examine species and treatment effects, followed by *t*-tests with Satterthwaite approximations to examine treatment effects for each pigweed species. Counts of plants with and without stem lesions were analyzed with asymptotic maximum-likelihood χ^2 tests.

Field experiment with *M. amaranthi*. Three summer and two fall trials were performed in 2003 and 2004 to evaluate the efficacy of *M. amaranthi*, the only pigweed bioherbicide permitted by the USDA-APHIS for field use in south Texas, against various pigweed species. There were four blocks, except where noted, of 1-m² field plots in each experiment, each plot containing 8 to 10 seedlings of a pigweed species with one inoculated and one control plot per block. Each plot was sprayed at a rate of 680 liter ha⁻¹ with a suspension of *M. amaranthi* or formulation control 1 week after transplanting 2-week-old greenhouse-grown pigweed seedlings. Each plot received 40 g of fertilizer (15-9-12 N-P-K plus micronutrients; Osmocote; Scotts-Sierra). Plots were irrigated at transplanting and twice per week thereafter. In a summer trial (2003) that involved *A. palmeri* and *A. hybridus*, *A. palmeri* plants

were 10.7 ± 0.5 cm tall with 13 ± 1 leaves and *A. hybridus* plants were 6.0 ± 0.3 cm tall with 6 ± 0.3 leaves. In the first fall (2003) trial with the same two pigweeds, *A. palmeri* plants were 29.8 ± 4.7 with 21.0 ± 1.7 leaves and *A. hybridus* plants were 30.8 ± 3.4 tall with 16.9 ± 1.1 leaves, while in the second fall (2004) trial, which involved a different local accession of *A. palmeri*, these plants were 18.7 ± 1.3 cm tall and had 29 ± 6 leaves, while *A. hybridus* plants were 16.6 ± 1.3 cm tall and had 30 ± 4 leaves. In two additional 2003 summer trials, *M. amaranthi* was applied to *A. retroflexus* (11.1 ± 0.4 cm tall, 17.2 ± 0.6 leaves) and *A. lividus* L. (livid amaranth) (4.5 ± 0.2 tall, 7.8 ± 0.3 leaves) in six and four blocks of plots, respectively. Number of leaves with necrosis and number of plants with stem lesions were counted after 2 weeks. Leaf and height gain were determined after 3 weeks. All measurements involved 3 to 10 plants per pigweed species per plot. Counts of leaves with necrosis and stems with lesions were summed and divided by total leaves or plants examined to obtain one percentage value for leaf necrosis incidence and stem lesion prevalence per plot. Leaf and height gains for individual plants were averaged within plots. Species, treatment, and species $\times$ treatment effects on ranked leaf necrosis and stem lesion percentages and log-transformed leaf and height gain were analyzed separately for each experiment involving two pigweed species using ANOVA with a random block effect. Satterthwaite *t*-tests were used to analyze treatment effects for each pigweed species when the species $\times$ treatment interaction was significant or when the trial involved a single species. Back-transformed leaf necrosis and stem lesion percentages are presented with confidence intervals.

Biochemical analyses. To investigate the responses in pigweed to infection with bioherbicidal fungi, four greenhouse experiments were conducted in which four to nine plants were treated with *M. amaranthi* or *P. amaranthicola* suspension or formulation control (23 *A. hybridus* and 16 *A. palmeri* per treatment). Samples of symptomatic leaves from inoculated plants and control leaves of the same age range (ap-

proximately 3 to 10 positions down from the shoot tip) were collected 2 weeks after inoculation and stored at -80°C . Soluble protein content was determined with bovine serum albumin as a standard, and the peroxidase activity level was determined with guaiacol as a substrate as described previously for pigweed (26). Samples (0.3 g) were homogenized in 0.01 M sodium phosphate buffer and centrifuged at 11,000 rpm for 15 min. Soluble protein content (milligrams per gram of fresh weight) and the peroxidase activity level (change in 1 min in absorbance per gram of fresh weight) were measured spectrophotometrically at 595 nm and 470 nm, respectively, using 6 and 150 μl of crude extract, respectively. FAA concentrations were determined in 0.5 g of ground frozen leaf tissue. Because of the amount of tissue required for the assay, tissues from four plants of each species-treatment combination were pooled into a single sample from each experiment and samples from two to four experiments were used in analyses. Samples were extracted in 10 ml 0.1 N HCl, centrifuged at 10,000 rpm for 30 min at 4°C , and syringe-filtered through 0.22 μm of polyvinylidene difluoride (Millipore, Milford, MA). FAA (picomole per microliter) were determined by reverse-phase high-pressure liquid chromatography (26) with an Agilent 1100 Series (Agilent Technologies, Atlanta, GA). Aspartic acid, glutamic acid, serine, threonine, alanine, and valine were detected at levels sufficient for comparing treatments. Species, treatment, and random experimental effects on log-transformed protein and peroxidase levels were examined with mixed-model ANOVA with subsequent *F*-tests for treatment effect tests in each pigweed species. Untransformed FAA levels were analyzed for species and treatment effects using ANOVA. Means ($\pm$ SE) of untransformed data are presented.

RESULTS

Greenhouse and field conditions.

Hourly temperatures were similar in the greenhouse and field between 3 September and 19 November in 2005 (daily average $\pm$ SE; $28.6 \pm 0.1^\circ\text{C}$, greenhouse; $26.3 \pm 0.1^\circ\text{C}$, field; $P = 0.104$), while maximum daily temperatures were higher in the greenhouse ($37.1 \pm 0.4^\circ\text{C}$) than in the field ($32.3 \pm 1.1^\circ\text{C}$) ($P < 0.0001$) (Fig. 1). Humidity was higher in the field ($75.1 \pm 0.5\%$) than in the greenhouse ($64.2 \pm 0.4\%$) ($P = 0.031$). In data collected from 1 September to 30 September 2005 under summer conditions, average daily greenhouse and field temperatures were similar ($29.7 \pm 0.2^\circ\text{C}$ and $29.1 \pm 0.2^\circ\text{C}$ average, respectively) ($P = 0.887$). Maximum daily temperatures were higher in the greenhouse ($39.0 \pm 0.5^\circ\text{C}$) than in the field ($35.2 \pm 0.5^\circ\text{C}$) ($P < 0.0001$), while daily average RH was higher in the field ($74.3 \pm 0.8\%$) than in the greenhouse ($71.1 \pm 0.6\%$) ($P <$

Fig. 1. Average hourly temperature (A) and relative humidity (B) over 78 days in a greenhouse used for inoculations of pigweeds with *Phomopsis amaranthicola* and *Microspphaeropsis amaranthi* and in a nearby field in the Lower Rio Grande Valley of Texas.

0.0001) (Fig. 1). During the hours when plants were kept under mist tents for one night (2000 to 0900 h) in September 2005, ambient humidity on subsequent nights was higher in the field ($88.0 \pm 1.3\%$) than in the greenhouse ($82.2 \pm 1.3\%$) ($P = 0.007$), while temperatures were similar ($26.6 \pm 0.1^\circ\text{C}$ and $26.4 \pm 0.2^\circ\text{C}$, respectively, $P = 0.388$). There was no natural dew period ($\text{RH} \geq 95\%$) in the greenhouse, while in the field, nightly dew periods lasted 5.1 ± 0.8 h.

Variation in susceptibility between pigweed species. No mortality occurred in any of the three pigweed species within 3 weeks of inoculation with either single or combined bioherbicides. *P. amaranthicola* and *M. amaranthi* produced punctate and vein leaf lesions, respectively, and each produced red-brown stem lesions. Leaf necrosis varied significantly by pathogen treatment ($P < 0.0001$), pigweed species ($P < 0.0001$), and the interaction of these factors ($P < 0.0001$) and inoculation led to significant leaf necrosis in two of the three species (Table 1). Across both single-pathogen treatments, necrosis incidence in *A. retroflexus* exceeded that in *A. hybridus*, which in turn exceeded that in *A. palmeri* ($P < 0.0001$). Inoculation with *P. amaranthicola* produced 20 and 90% more leaves with necrosis on *A. hybridus* and *A. retroflexus*, respectively, than on *A. palmeri*, while inoculation with *M. amaranthi* led to 26 and 92% more necrotic leaves in *A. hybridus* and *A. retroflexus*, respectively, than in *A. palmeri* (Table 1). Inoculation with combined pathogens did not increase leaf necrosis incidence over single-pathogen treatments. Means of leaf necrosis incidence varied widely among experiments in *A. hybridus* (control, 0 to 25.2%; *P. amaranthicola*, 0 to 76.9%; *M. amaran-*

thi, 3.0 to 54.1%) and *A. retroflexus* (control, 0 to 8.8%; *P. amaranthicola*, 5.9 to 92.6%; *M. amaranthi*, 10.3 to 97.3%). In *A. palmeri*, means for the single-pathogen treatments and the control ranged from 0 to 10.2% leaf necrosis incidence.

The prevalence of plants with stem lesions varied significantly by pathogen treatment ($P = 0.035$) and between pigweed species ($P < 0.0001$). Stem lesions were 28 and 95% more prevalent in *A. hybridus* and *A. retroflexus*, respectively, than in *A. palmeri* after *P. amaranthicola* inoculation, and 54% more *A. hybridus* and 78% more *A. retroflexus* plants developed at least one stem lesion after *M. amaranthi* inoculation than did *A. palmeri* (Table 1). Combined-pathogen inoculation increased stem lesion incidence by 45% in *A. hybridus* and 8% in *A. palmeri* compared with controls, but did not enhance lesion incidence over single-pathogen treatment in either species (Table 1). Stem lesion incidence varied among experiments in *A. hybridus* (control, 0 to 5%; *P. amaranthicola*, 0 to 100%; *M. amaranthi*, 0 to 100%) and *A. retroflexus* (control, 0%; *P. amaranthicola*, 80 to 100%; *M. amaranthi*, 75 to 100%), while ranging from 0 to 8% in all treatments in *A. palmeri*.

Leaf gain varied by pigweed species ($P < 0.0001$), with control *A. palmeri* plants gaining twofold more leaves than control *A. hybridus* and 7.5-fold more leaves than control *A. retroflexus* over 3 weeks (Table 1). Leaf gain did not vary by pathogen treatment across all three species ($P = 0.223$). Although the species $\times$ treatment interaction was also not significant ($P = 0.157$), analysis of results for each pigweed species separately revealed that single- and combined-pathogen treatments reduced leaf gain by 24 to 36% in *A. hy-*

bridus (Table 1). Leaf gain varied by experiment in *A. hybridus* (control, 10.8 to 37.2 leaves; *P. amaranthicola*, 6.4 to 28.2 leaves; *M. amaranthi*, 8.6 to 25.4 leaves), *A. retroflexus* (control, 0 to 39.8 leaves; *P. amaranthicola*, 0 to 44.2 leaves; *M. amaranthi*, 0 to 31.0 leaves) and *A. palmeri* (control, 23.6 to 84.8 leaves; *P. amaranthicola*, 14.6 to 76.6 leaves; *M. amaranthi*, 16.4 to 106.2 leaves).

Plant height gain was not influenced by treatment ($P = 0.807$) or by the treatment $\times$ species interaction ($P = 0.202$) but height gains differed between pigweed species ($P < 0.0001$). For example, height gain in *A. palmeri* control plants (44.4 ± 3.9 cm) was considerably greater than in *A. hybridus* (18.7 ± 1.2 cm) or *A. retroflexus* (9.1 ± 3.5 cm) controls.

Efficacy in young and mature pigweeds. In young plants, leaf necrosis incidence varied by pathogen treatment ($P < 0.0001$) and pigweed species ($P < 0.0001$) but not according to the treatment-species interaction ($P = 0.088$). Young inoculated *A. hybridus* and *A. retroflexus* plants had 11 and 13% more leaves with lesions, respectively, than did *A. palmeri* (Table 2). Inoculation increased leaf lesion incidence by 5% in *A. palmeri*, in contrast to the prior set of greenhouse experiments. The incidence of plants with stem lesions was influenced by treatment ($P = 0.048$) and pigweed species ($P < 0.0001$). Stem lesions occurred in inoculated *A. hybridus* and *A. retroflexus* but not in *A. palmeri*. Final leaf counts were not influenced by treatment across all three pigweed species ($P = 0.487$) but was affected by plant species ($P < 0.0001$) and the treatment $\times$ species interaction ($P = 0.030$). Three weeks after inoculation, young control *A. palmeri* plants had more leaves than did *A. hy-*

Table 1. Effects of inoculation of *Amaranthus palmeri* (Ap), *A. hybridus* (Ah), and *A. retroflexus* (Ar) pigweeds with a single fungal pathogen (*Phomopsis amaranthicola* or *Microsphaeropsis amaranthi*) or combined pathogens

Effect	Inoculation treatment ^v				F or χ^2	P
Species	Control	<i>P. amaranthicola</i>	<i>M. amaranthi</i>	Both		
Leaf necrosis (%) ^w						
Ap	0.5 (0-1.3) a	0.6 (0.1-1.6) a	1.4 (0.4-3.0) a	1.3 (0.1-3.7) a	1.38	0.251
Ah	5.0 (2.7-8.5) b	21.0 (13.6-29.9) a	27.9 (22.2-34.2) a	28.5 (22.9-34.6) a	53.8	<0.0001
Ar	1.7 (0.1-5.0) b	92.2 (68.6-99.4) a	93.5 (67.6-97.7) a	ND ^x	77.5	<0.0001
Stem lesions (%) ^y						
Ap	0 a	0 a	3 a	8 a	4.53	0.210
Ah	2 b	27 a	57 a	46 a	49.7	<0.0001
Ar	0 b	95 a	81 a	ND	57.7	<0.0001
Net leaf gain ^z						
Ap	44.8 ± 5.3 a	44.1 ± 4.8 a	40.6 ± 6.1 a	30.2 ± 3.5 a	0.88	0.455
Ah	22.5 ± 1.6 a	15.5 ± 1.9 b	16.6 ± 1.4 b	14.2 ± 4.4 b	4.63	0.004
Ar	6.0 ± 2.6 a	3.9 ± 2.1 a	10.3 ± 4.3 a	ND	1.70	0.190

^v Results for single-pathogen and control treatments on *A. palmeri* and *A. hybridus* are means of seven experiments. Results for single-pathogen and control treatments on *A. retroflexus* are means of three of these experiments. One of the experiments involved combined-pathogen inoculation of *A. palmeri* and *A. hybridus*. Numbers of plants varied among species-treatment combinations and are presented in Materials and Methods.

^w Percentage of leaves per plant with leaf necrosis (average with lower-upper 95% confidence intervals). Means followed by different letters within each row are significantly different ($P = 0.05$) based on Tukey mean comparisons.

^x ND = not determined.

^y Percentage of plants per treatment with at least one stem lesion. The effect of pathogen treatment was analyzed using a likelihood-ratio χ^2 test. A different letter following treatment and control data indicates significantly higher stem lesion incidence ($P = 0.05$) in that treatment than in the control.

^z Leaf gain per treatment (average $\pm$ SE). Means followed by different letters within each row are significantly different ($P = 0.05$) in Tukey mean comparisons.

bridus and *A. retroflexus* (1.3- and 1.2-fold differences, respectively). A significant reduction (32%) in leaves occurred only in inoculated *A. hybridus* (Table 2). Final heights (3 weeks after inoculation, plants were 39 days old) varied across species ($P < 0.0001$), with control *A. palmeri* (44.7 ± 4.9 cm) being 1.6-fold taller than *A. hybridus* (27.9 ± 1.7 cm) but statistically similar to *A. retroflexus* (35.2 ± 4.0 cm). Heights were not influenced by treatment ($P = 0.445$) or the treatment $\times$ species interaction ($P = 0.533$). Combined inoculation did not lead to mortality in young plants.

In mature plants, leaf necrosis varied between treatments ($P < 0.0001$), pigweed species ($P < 0.0001$), and their interaction ($P < 0.0001$). Inoculated *A. hybridus* and *A. retroflexus* plants had 27 and 89% more necrotic leaves, respectively, than did inoculated *A. palmeri* plants, which had only 4% more necrotic leaves than the control (Table 2). Stem lesion prevalence did not vary by treatment across all three pigweed species ($P = 0.481$) but did vary by species ($P < 0.0001$). Inoculated *A. hybridus* and *A. retroflexus* had 63 and 56% more plants with stem lesions, respectively, than did *A. palmeri*, in which only 8% of inoculated plants developed one or more lesions (Table 2). Final leaf counts did not vary by treatment ($P = 0.234$) or treatment $\times$ pigweed species interaction ($P = 0.105$) but did vary by species ($P < 0.0001$). The *A. palmeri* control had twofold more leaves than *A. hybridus* and 3.7-fold more leaves than *A. retroflexus*. Leaf abscission negatively influenced leaf counts in all mature control plants, especially *A. retroflexus*. Leaf gain was reduced 16% by inoculation in *A. hybridus* (Table 2). Final heights in mature plants were significantly influenced by pigweed species ($P < 0.0001$) but not by treatment ($P = 0.422$) or treatment $\times$ species interaction ($P = 0.678$). Control *A. palmeri* plants were 1.6-fold taller than *A. hybridus* controls and 2.3-fold taller than *A. retroflexus* controls.

Efficacy of *M. amaranthi* in the field.

Daily average temperatures were higher in the summer ($29.2 \pm 0.2^\circ\text{C}$) than in the fall ($17.5 \pm 0.6^\circ\text{C}$, $P < 0.001$) field season, as were daily maximum temperatures ($35.4 \pm 0.3^\circ\text{C}$ summer, $23.5 \pm 0.7^\circ\text{C}$ fall, $P < 0.0001$) and 2000 to 0900 h nighttime temperatures ($26.6 \pm 0.2^\circ\text{C}$ summer, $14.9 \pm 0.6^\circ\text{C}$ fall, $P < 0.0001$). Daily average RH was similar between seasons ($77.7 \pm 0.9\%$ summer, $73.2 \pm 0.7\%$ fall, $P = 0.135$), as was nighttime RH ($90.8 \pm 0.6\%$ summer, $84.0 \pm 2\%$ fall, $P = 0.101$), and the duration of natural dew periods (7.1 ± 0.5 h in summer, 6.8 ± 0.7 h in fall, $P = 0.698$).

In summer trial 1 (Table 3), pathogen treatment ($P = 0.026$) and pigweed species ($P < 0.0001$) influenced leaf necrosis incidence but not the treatment $\times$ species interaction ($P = 0.152$). Across both species, the incidence of necrotic leaves was not significantly higher in plots inoculated with *M. amaranthi* than in control plots, but necrosis incidence was higher on *A. hybridus* than on *A. palmeri*, irrespective of treatment (Table 3). The prevalence of stem lesions varied by treatment ($P = 0.0001$), pigweed species ($P = 0.003$), and their interaction ($P = 0.003$). Stem lesions were 80% more prevalent in inoculated *A. hybridus* than in inoculated *A. palmeri* (Table 3). *A. hybridus* and *A. palmeri* differed in leaf and height gain ($P < 0.0001$ and 0.0004 , respectively). Control *A. palmeri* plants gained 139 ± 5.8 leaves and 81.5 ± 4.6 cm in height compared with 32.2 ± 3.4 leaves and 30.1 ± 4.1 cm in height in *A. hybridus* (data not presented). Pathogen treatment did not influence leaf or height gain ($P = 0.200$ and 0.147 , respectively) nor did the treatment $\times$ species interaction ($P = 0.194$ and 0.316).

In the first of two fall field trials involving *A. palmeri* and *A. hybridus*, pathogen treatment ($P < 0.0001$), pigweed species ($P = 0.0007$), and their interaction

($P = 0.0007$) all influenced the incidence of necrotic leaves, while in the second trial, treatment ($P = 0.001$) and species ($P = 0.018$) effects were significant but their interaction was not ($P = 0.254$). Inoculation with *M. amaranthi* significantly increased (by 6 to 17%) the incidence of leaves with necrosis in *A. hybridus* over controls in both trials. Leaf necrosis incidence increased 10 to 11% after inoculation of *A. palmeri* (Table 3). The prevalence of plants with stem lesions varied by pathogen treatment in both the first ($P = 0.019$) and second ($P = 0.004$) fall trials, but did not vary by pigweed species ($P = 0.331$, $P = 0.229$, respectively) or by the treatment $\times$ species interaction ($P = 0.331$, $P = 0.401$, respectively) in either trial. Inoculation increased the prevalence of *A. hybridus* plants with stem lesions by 75 and 23% in the first and second fall field trials, respectively, compared with controls. In the second trial, stem lesion prevalence increased by 64% in *A. palmeri* after inoculation (Table 3). Inoculation did not influence leaf gain in the first ($P = 0.756$) or second ($P = 0.274$) fall trial, nor did pigweed species ($P = 0.351$, $P = 0.873$, respectively) or the treatment $\times$ species interaction ($P = 0.674$, $P = 0.758$) (data not presented). Height gain was marginally influenced by treatment in the first trial ($P = 0.051$), with inoculated *A. palmeri* plants gaining 14 cm more in height than control plants, but there was no treatment effect in the second trial ($P = 0.377$), and pigweed species ($P = 0.967$, $P = 0.118$) and the species $\times$ treatment interaction ($P = 0.087$, $P = 0.377$) had no effects in either trial. In the second fall trial, control *A. palmeri* plants gained 13.3 ± 3.8 leaves and 6.0 ± 3.4 cm in height, while *A. hybridus* controls gained 10.8 ± 1.5 leaves and 1.9 ± 1.0 cm in height.

In summer trial 2, involving *A. retroflexus*, control plants had 9% more necrotic leaves than *M. amaranthi*-inoculated plants and no stem lesions developed in

Table 2. Effects of inoculation of young and mature *Amaranthus palmeri* (Ap), *A. hybridus* (Ah), and *A. retroflexus* (Ar) pigweeds with combined *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* pathogens

Effect Species	Young plants ^v				Mature plants ^w			
	Control	Inoculated	<i>T</i> or χ^2	<i>P</i>	Control	Inoculated	<i>t</i> or χ^2	<i>P</i>
Leaf necrosis(%) ^x								
Ap	0.2 (0-2.9)	4.9 (2.5-8.0)	3.58	0.001	0.03 (0-0.04)	4.2 (1.1-9.4)	3.89	0.002
Ah	1.1 (0.1-3.5)	16.4 (12.0-21.6)	6.30	<0.0001	1.4 (0.2-3.5)	32.8 (29.1-36.6)	9.27	<0.0001
Ar	1.5 (0.1-4.7)	18.2 (12.9-24.2)	6.03	<0.0001	0.4 (0.0-2.3)	92.5 (80.7-99.3)	13.6	<0.0001
Stem lesions (%) ^y								
Ap	0	0	...	...	0	8	1.51	0.219
Ah	0	60	16.5	<0.0001	7	71	13.6	0.0002
Ar	0	27	6.16	0.013	0	64	16.1	<0.0001
Number of leaves ^z								
Ap	37.7 ± 3.9	50.1 ± 7.5	1.27	0.216	47.8 ± 7.5	60.3 ± 8.0	1.07	0.295
Ah	29.7 ± 2.2	22.3 ± 2.6	2.27	0.032	36.3 ± 1.6	30.6 ± 1.7	2.42	0.023
Ar	32.9 ± 3.9	30.8 ± 3.6	0.20	0.841	12.8 ± 1.2	16.3 ± 1.2	1.87	0.073

^v Results are means of three experiments on young (18-day-old) plants.

^w Results from one experiment on mature (35-day-old) plants.

^x Percentage of leaves per plant with leaf necrosis (average with lower-upper 95% confidence intervals). *T*-tests were used to analyze treatment effects.

^y Percentage of plants per treatment with at least one stem lesion. The effect of pathogen treatment was examined with a likelihood-ratio χ^2 test.

^z Final leaf counts (average $\pm$ SE). *T*-tests were used to analyze treatment effects.

response to *M. amaranthi* (Table 3). In summer trial 3 on *A. lividus*, inoculation did not increase leaf necrosis incidence, but most plants in the inoculated plots developed stem lesions (Table 3). Inoculation with *M. amaranthi* did not influence leaf or height gain in either of these two experiments ($P > 0.05$).

Biochemical responses to inoculation. Protein content in *A. hybridus* and *A. palmeri* varied marginally in response to inoculation with *P. amaranthicola* or *M. amaranthi* ($P = 0.069$) and protein content was 18% lower in *M. amaranthi*-inoculated *A. palmeri* than in control

plants (Table 4), but protein content did not vary between pigweed species ($P = 0.430$). Means for protein content, measured as milligrams per gram of fresh weight, varied across experiments for *A. palmeri* (control, 8.3 to 11.3; *P. amaranthicola*, 6.8 to 13.7; *M. amaranthi*, 5.1 to 11.7) and *A. hybridus* (control, 6.3 to 14.5; *P. amaranthicola*, 9.2 to 11.9; *M. amaranthi*, 7.8 to 9.8). The peroxidase activity level varied by pathogen treatment ($P = 0.048$) and species ($P < 0.0001$). *P. amaranthicola* increased the soluble peroxidase activity level 1.8-fold in *A. palmeri* (Table 4), while neither of the two patho-

gens influenced the soluble peroxidase activity level in *A. hybridus*. The range of means for peroxidase (change in absorbance per gram of fresh weight in 1 min) across experiments were: *A. palmeri*, control, 3.2 to 35.1; *P. amaranthicola*, 5.6 to 97.7; *M. amaranthi*, 9.4 to 34.7; and in *A. hybridus*, control, 4.3 to 25.4; *P. amaranthicola*, 5.0 to 32.3; *M. amaranthi*, 6.2 to 21.4. Neither aspartic nor glutamic acid FAA levels varied by treatment ($P = 0.554$, $P = 0.882$, respectively) or treatment $\times$ species interaction ($P = 0.391$, $P = 0.785$), but both varied by species. Aspartic acid was 2.5-fold greater in *A. hybridus* ($P =$

Table 3. Effects of inoculation of *Amaranthus palmeri* (Ap), *A. hybridus* (Ah), *A. retroflexus* (Ar), and *A. lividus* (Al) pigweeds with *Microsphaeropsis amaranthi* in summer and fall field plot experiments^w

Trial	Effect	Species	Control	<i>M. amaranthi</i>	<i>t</i>	<i>P</i>
Summer trials						
1	Leaf necrosis (%) ^x	Ap	0.2 (0.1-1.7)	2.8 (1.0-5.5)	... ^y	...
		Ah	17.2 (7.5-30.7)	25.8 (6.5-55.6)	...	...
		Mean	5.3 (0.3-16.4)	11.5 (2.7-26.5)	1.05	0.311
	Stem lesions (%) ^z	Ap	0	1.7 (0-29.5)	1.00	0.391
		Ah	0	82.1 (7.3-100)	11.2	0.002
2	Leaf necrosis (%)	Ar	15.9 (8.1-26.2)	6.4 (1.1-16.0)	2.52	0.032
	Stem lesions (%)	Ar	0	0	...	...
3	Leaf necrosis (%)	Al	1.4 (0.2-3.5)	0.5 (0-2.3)	1.59	0.170
	Stem lesions (%)	Al	0	99.7 (51.7-100)	6.53	0.007
Fall trials						
1	Leaf necrosis (%)	Ap	0	10.3 (7.4-13.7)	9.30	0.0001
		Ah	0	17.3 (13.6-21.5)	15.5	<0.0001
		Mean	2.4 (0.7-15.3)	45.5 (6.4-95.1)	2.70	0.020
	Stem lesions (%)	Ap	2.3 (0-40.2)	5.7 (0-83.9)	...	...
		Ah	2.3 (0-40.2)	76.9 (4.8-100)	...	...
2	Leaf necrosis (%)	Mean	2.4 (0-8.6)	13.6 (9.8-18.0)	3.50	0.004
		Ap	0	11.9 (3.5-20.9)	...	...
		Ah	9.5 (6.9-12.5)	15.3 (8.4-24.3)	...	...
	Stem lesions (%)	Mean	13.8 (1.1-39.6)	62.2 (27.8-94.1)	2.96	0.011
		Ap	2.4 (0-40.2)	66.5 (0-100)	...	...
		Ah	34.0 (14.4-59.4)	57.8 (20.0-95.4)	...	...
		Mean	13.8 (1.1-39.6)	62.2 (27.8-94.1)	2.96	0.011

^w All trials involved four blocks except for summer trial 2, which involved six blocks.

^x Percentage of leaves per plot with leaf necrosis (average with lower-upper 95% confidence intervals).

^y ... Denotes omission of a *t*-test and *P* determination for the treatment effect because the treatment $\times$ species interaction was not significant except for stem lesions in summer trial 2, in which no statistical test could be run.

^z Percentage of plants per plot with at least one stem lesion (average with lower-upper 95% confidence intervals).

Table 4. Effects of inoculation of *Amaranthus palmeri* (Ap) and *A. hybridus* (Ah) with *Phomopsis amaranthicola* or *Microsphaeropsis amaranthi* on protein content, peroxidase activity level, and aspartic and glutamic free amino acid content

	Species	Control	<i>P. amaranthicola</i>	<i>M. amaranthi</i>	<i>F</i>	<i>P</i>
Protein ^x	Ap	9.57 $\pm$ 0.55 a	9.76 $\pm$ 1.00 a	7.82 $\pm$ 0.80 b	3.73	0.033
	Ah	8.55 $\pm$ 0.89 a	10.7 $\pm$ 0.77 a	9.04 $\pm$ 0.42 a	2.24	0.116
Peroxidase ^y	Ap	27.0 $\pm$ 5.33 b	49.1 $\pm$ 13.8 a	28.2 $\pm$ 4.24 b	3.24	0.049
	Ah	15.1 $\pm$ 3.58 a	20.6 $\pm$ 4.38 a	12.8 $\pm$ 2.47 a	0.57	0.570
Aspartic acid ^z	Ap	307 $\pm$ 87 a	339 $\pm$ 94 a	526 $\pm$ 13 a	1.61	0.289
	Ah	774 $\pm$ 99 a	586 $\pm$ 175 a	668 $\pm$ 112 a	0.56	0.595
Glutamic acid ^z	Ap	1,002 $\pm$ 63 a	1,216 $\pm$ 256 a	1,014 $\pm$ 88 a	0.47	0.649
	Ah	450 $\pm$ 162 a	463 $\pm$ 164 a	619 $\pm$ 352 a	0.14	0.872

^x Soluble protein content (average $\pm$ SE) as milligrams per gram of fresh weight. Results are means of four greenhouse experiments. Means followed by different letters within each row are significantly different ($P \leq 0.05$) in Tukey mean comparisons.

^y Soluble peroxidase activity level (average $\pm$ SE) as the change in absorbance in 1 min per gram of fresh weight. Results are means of four greenhouse experiments. Means followed by different letters within each row are significantly different ($P \leq 0.05$) in Tukey mean comparisons.

^z Free amino acid content (average $\pm$ SE) as picomoles per microliter. Results are means of two to four samples per treatment per species. Each sample was a pool of leaf tissue from one experiment.

0.011) and glutamic acid was 2.2-fold greater in *A. palmeri* ($P = 0.015$). Inoculation did not influence concentrations of alanine, serine, threonine, and valine (data not presented).

DISCUSSION

Greenhouse conditions for inoculations with pigweed bioherbicides reflected summer field conditions in south Texas. However, the higher maximum daily temperatures, lower average humidity, and absence of a 'natural' dew period may have made greenhouse conditions more limiting to infection than those in the field. In neither environment were pigweeds killed by inoculation with *P. amaranthicola*, *M. amaranthi*, or combined pathogens, which is in contrast to past studies (29,33,35). Average greenhouse and summer field temperatures were 2 to 9°C higher and ambient humidity as much as 10% lower in this study than in some prior field studies, (29,33,44). Comparisons of temperature and rainfall data from Weslaco, TX (28) with data from previous testing locations (Gainesville, FL and Urbana, IL) support the conclusion that ambient temperatures were higher and the humidity lower in south Texas.

Germination and initial penetration by conidia of both fungi occur between 15°C (*M. amaranthi*) or 20°C (*P. amaranthicola*) and 30°C (30,44). The infection process takes at least 15 h and is not always complete by 24 h (30). In greenhouse and field experiments in this study, temperature conditions during the first 13 h after inoculation, when plants were under the mist tents, were compatible with conidial germination and germ tube elongation of both pathogens on leaf surfaces (30), although high compared with some past studies on *M. amaranthi* (44). The 13-h dew period used in this study was sufficient to produce mortality in other studies on *P. amaranthicola* (35), *M. amaranthi* (44), and on other pigweed pathogens (12,19). Similar dew periods (10 to 15 h) were used in bioherbicide trials on waterhyacinth (*Eichhornia crassipes* Solms-Laubach), kudzu (*Pueraria montana* [Lour.] Merr. var. *lobata* [Willd.] Massen & S.M. Almeida), and dandelion (*Taraxacum officinale* Weber (7,9,13,37,38,40,50). The relatively low incidence of disease and lack of mortality observed in greenhouse experiments were thus likely related to high maximum temperatures and variable, at times low, humidity that occurred after the dew period. (29,33). The role of environmental limitations in this study was therefore restricted to events occurring after fungal penetration. The incidence of leaf necrosis and stem lesions would have likely been still lower in the absence of the artificially imposed initial dew period. Improved formulations, containing oils and other ingredients in addition to or in place of the surfactant and humectant used in this study, could decrease humidity requirements and

increase mortality on pigweeds (15,23,25), as in other bioherbicide systems (2,3,7,19,37,40,45,50).

In greenhouse experiments, despite a wide degree of variation in symptom incidence between experiments, *A. retroflexus* and *A. hybridus* showed greater susceptibility to infection by *P. amaranthicola* and *M. amaranthi* than did *A. palmeri*. Net leaf gain was reduced only in *A. hybridus*, likely due to leaf abscission (29), while gains in shoot height were never negatively affected. Inoculation with combined pathogens did not specifically increase symptom incidence over single-pathogen treatment, in agreement with past symptom and mortality results for these two bioherbicides (29). Our results corroborate initial work on *M. amaranthi*, which characterized *A. palmeri*, at the four- to six-leaf stage, as resistant and *A. hybridus* and *A. retroflexus* as susceptible (24). In testing *P. amaranthicola*, Roskopf et al. (34) placed most of the *A. retroflexus* and *A. hybridus* accessions they tested in higher mortality classes than *A. palmeri*. Ortiz-Ribbing and Williams (29) reported more severe effects of *P. amaranthicola* on biomass and mortality in *A. hybridus* than in *A. palmeri* in the field, but also demonstrated the ability of *M. amaranthi* and combined-pathogen application in Illinois to kill all three of the *Amaranthus* spp. tested in south Texas in this study. Conidial germination of *P. amaranthicola* and *M. amaranthi* were similar on the three pigweed species, and germ tube elongation was actually greatest on *A. palmeri* (30). Interspecific variation in resistance can emerge in later stages of the infection process (4,21). Symptom incidence, but not symptom development (i.e., leaf area exhibiting symptoms over time), were measured in this study, and so the roles of resistance in influencing pre- versus post-penetration events and subsequent symptom levels are not clear. The fast growth of *A. palmeri*, as evidenced in this study by higher net leaf and shoot height gains in *A. palmeri* control plants than in controls of the other *Amaranthus* spp., could have allowed *A. palmeri* to tolerate infection, further reducing the efficacy of the two bioherbicides against this regionally dominant pigweed species.

The incidence of leaf necrosis and stem lesions in this study in relatively susceptible *A. hybridus* was below levels reported in earlier studies (29,33,35). Some previous studies allowed more time (5 to 8 weeks) to assess disease development and mortality, although 15 days was sufficient for mortality to occur in all three pigweed species in one study (29). Weed age has been reported to be a critical factor in determining the efficacy of fungal bioherbicides, with mortality being highest in young (one- to six-leaf) plants (15), typically used in studies of *P. amaranthicola* and *M. amaranthi* (29,33,34,44,45) and *Alternaria alternata* (Fr.) Keissler (12,19)

on *Amaranthus* spp. Roskopf et al. (35) reported as much as 100% mortality 8 weeks after *P. amaranthicola* inoculation of four- to six-leaf *A. hybridus* incubated for 8 h at a dew temperature of 35°C and then maintained in a greenhouse averaging 35°C during the day. Plant age may thus be as important as environmental conditions in limiting efficacy. The young plants used in this study were large relative to past studies, having 4 to 10 leaves in greenhouse and summer field experiments and as many as 30 leaves in fall field experiments. Only one greenhouse experiment was conducted with mature plants and young plants were tested separately. Nonetheless, the results suggest that young and mature (35-day-old) plants of *A. hybridus* and *A. retroflexus* exhibited roughly similar disease incidence levels that in both cases exceeded levels on *A. palmeri* plants of comparable ages. The modest negative effect on *A. hybridus* leaf counts was also common to both ages. Our results suggest that sublethal disease incidence after *P. amaranthicola* and *M. amaranthi* inoculation is affected less by weed age than by interspecific differences in susceptibility. Symptom development on mature *A. hybridus* and *A. retroflexus* could contribute to weed management in the field if mature infected plants act as inoculum reservoirs for nearby seedlings or if infection of mature, flowering plants reduces reproduction.

Average summer and fall temperatures and humidity in the field were likely suitable for fungal infection, but, as in the comparisons of greenhouse with field environments, environmental extremes (maximum temperatures in the summer, minimums in the fall) may have been more important than averages. In the fall field trial, low temperatures (below 15°C) during the dew period and on subsequent nights could have limited symptom incidence in plants infected with *M. amaranthi* (35,44). In one summer field trial and in the first fall field trial with *M. amaranthi*, inoculated *A. hybridus* plants showed symptom incidence levels similar to those observed in the greenhouse. However, abiotic (wind, sand) or biotic (insects) damage may have obscured inoculation effects on *A. hybridus* in the summer trial and in the second fall trial. The high incidence of stem lesions in inoculated *A. palmeri* in the second fall field trial may have been related to the use of a separate accession for this trial. Leaf and height gain were not greater in *A. palmeri* than in *A. hybridus* in the two fall trials, in contrast to the results from greenhouse experiments and summer field trials. Reduced fall growth may have increased leaf necrosis symptom incidence on *A. palmeri*. The lack of symptoms in *A. retroflexus* in one summer trial are suggestive of a procedural error, such as mist tent failure, rather than environmental constraints since inocula-

tion increased leaf and stem necrosis incidence in *A. hybridus* under similar field conditions and in *A. retroflexus* in the greenhouse. The increase in stem lesion incidence, though not leaf necrosis, in *A. lividus* is consistent with the known susceptibility of this species to *M. amaranthi* (23), and *A. lividus* can also be killed by *P. amaranthicola* (29,34).

Low symptom incidence in *A. palmeri* after *P. amaranthicola* inoculation may have been related to peroxidase induction, a common response to pathogen infection (21,47). The herbicide bentazon increased dry mass losses in velvetleaf (*Abutilon theophrasti* Medik) inoculated with *Colletotrichum coccodes* (Wallr.) Hughes (1), in part by suppressing peroxidase induction by twofold. Bentazon is compatible with *P. amaranthicola* germination and growth (49) and could reduce the twofold peroxidase induction that occurred in *A. palmeri*, potentially leading to greater symptom development in response to *P. amaranthicola*. Other bioherbicide-chemical herbicide combinations could increase the efficacy of *M. amaranthi* (45), as in other bioherbicide systems (38).

Protein and FAA content are commonly altered by fungal infection (16), possibly affecting feeding and growth of pest insects (48). The beet armyworm (*Spodoptera exigua* Hübner), a pest of cotton, uses pigweeds as hosts (26,41) and might show different feeding patterns on *M. amaranthi*-infected and healthy *A. palmeri* because of reductions in soluble protein after inoculation. Changes in FAA accumulations in pigweeds (26) and other plants (27,42,43) are associated with plant stresses, including water deficit and infection by pathogens. The stability of the six FAA detected in our study suggests that *P. amaranthicola* and *M. amaranthi* infection do not induce water deficit stress responses in pigweeds. However, environmental factors, plant phenology, interspecific resistance variation, and physiochemical induction all influence the efficacy of these two pathogens on *Amaranthus* spp.

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