

# Factors Influencing the Biocontrol of Tumble Pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*

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## ABSTRACT

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Growth chamber studies and field tests were conducted to evaluate *Aposphaeria amaranthi* as a potential mycoherbicide for tumble pigweed (*Amaranthus albus*). Seedlings in growth chamber studies were killed within 2 days after inoculation with  $1 \times 10^6$  conidia per milliliter and an 8-hr dew period at 28 C. The onset of dew could be delayed for 72 hr postinoculation without an apparent decrease in disease severity. Inoculations with conidial concentrations as low as  $1 \times 10^4$ /ml were sufficient for plant death when followed by a 24-hr dew period. Dew temperatures from 20 to 28 C were conducive for disease development. In field tests, 99% of seedlings with two to six true leaves were killed when sprayed to runoff with  $6 \times 10^6$  conidia per milliliter. Results from these studies suggest that *A. amaranthi* may have potential as a mycoherbicide for tumble pigweed.

Additional keyword: bioherbicide

The genus *Amaranthus* includes over 60 known species of broad-leaved plants. Some species are used for grain and ornamentals, but the majority are characterized as weeds (15), and some are considered among the world's worst weeds (8). *Amaranthus albus* L., known as tumble pigweed, is common throughout the United States in cultivated or fallow fields (9). Pigweed traditionally has been controlled with chemical herbicides (10). However, pigweed populations resistant to the triazine herbicides have been reported in the United States and Canada (1,7,18), and triazine-resistant weeds may also be cross-resistant to other herbicides (6). As trends toward decreased tillage systems increase, problems with herbicide resistance may become more serious (7), creating a need for alternative control methods.

In 1987, a fungus identified as *Aposphaeria amaranthi* Ell. & Barth. was isolated from diseased stem tissue of an unknown species of pigweed collected at the Agricultural Experiment Station Farm at the University of Arkansas, Fayetteville. *A. amaranthi* was first described from redroot pigweed (*A. retroflexus* L.) in 1896 (5) and listed by

Saccardo in 1899 (16). No further information about this fungus has been reported in the literature. Preliminary pathological studies demonstrated pathogenicity of *A. amaranthi* to several different *Amaranthus* spp., including spiny pigweed (*A. spinosus* L.), redroot pigweed, tumble pigweed, and careless weed (*A. hybridus* L.). The purpose of the growth chamber studies was to determine the influence of dew temperature, plant age, conidial concentration, dew period, and dew period delay on the efficacy of *A. amaranthi* as a biological control for tumble pigweed. Field tests were conducted to determine the conidial concentrations and application rates necessary for plant mortality.

## MATERIALS AND METHODS

Stem tissues of a diseased pigweed plant were surface-disinfested by immersion in 1% sodium hypochlorite for 30 sec, followed by a rinse in sterile water for 1 min. Tissue pieces were then transferred to potato-dextrose agar (PDA) plates amended with 0.3 mg/ml of streptomycin sulfate and incubated at room temperature (23 C). Sporulating isolates were transferred to fresh PDA plates, and conidia from selected isolates were stored at -80 C in a 1:1 skim milk/glycerol solution. The identity of the causal agent was confirmed by B. C. Sutton (C.A.B International Mycological Institute, IMI 327754). Conidia of *A. amaranthi* used for inoculations were obtained by subculturing the fungus on pea-juice agar plates (19) from cultures in cryogenic storage. The cultures were incubated under fluorescent lights (12-hr photoperiod) at 24-26 C for 3-5 days, and conidia were rinsed from the

plates with distilled water and strained through a 1-mm mesh screen. Each petri plate yielded approximately 50 ml of inoculum at a concentration of  $1-2 \times 10^6$  conidia per milliliter. Conidial concentrations were determined with a hemacytometer.

**Growth chamber studies.** Plants of tumble pigweed were grown from seed in round, 8-cm-diameter plastic pots containing vermiculite in a 28 C growth chamber (12-hr photoperiod,  $330 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and were fertilized weekly. After emergence, seedlings were thinned to three to five plants per pot. Seedlings were inoculated at the four- to six-true-leaf stage, except for those used in the plant age study.

Plants were sprayed to runoff (approximately 3 ml per plant) with  $1-2 \times 10^6$  conidia per milliliter unless otherwise stated. With the exception of the delayed dew period test, plants were transferred immediately after inoculation to a dark dew chamber. Seedlings were given a 24-hr dew period at 28 C unless indicated otherwise. After the dew period, plants were returned to the 28 C growth chamber.

Disease severity and plant mortality were determined 2 and 10 days after inoculation. Disease ratings and percent mortality were averaged for all plants in a pot. Each treatment consisted of at least three replicated pots, each with three to five plants. Controls for each experiment consisted of two pots sprayed with distilled water. Each experiment was repeated at least twice. Disease severity was assessed on a rating system of 0-5, in which 0 = no visible symptoms, 1 = <25% leaf necrosis with no stem lesions, 2 = 26-50% total plant necrosis, 3 = 51-75% total plant necrosis, 4 = 76-99% total plant necrosis, and 5 = plant death. Seedling mortality was determined by counting the number of dead plants in each pot. Data were analyzed by an analysis of variance in which the sources of variation were the test, the variable, and the interaction. Because the interaction was nonsignificant ( $P > 0.2$ ), the interaction and residual were pooled and used as the error terms in subsequent analysis. The hour or age source was partitioned into linear ( $P < 0.001$ ), quadratic ( $P < 0.001$ ), and residual sources. The residual was also nonsignificant ( $P > 0.2$ ). Treatment means were compared using the least significant difference at the 5% significance level.

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The effect of temperature during the dew period was evaluated at 20, 24, 28, and 32 C. Each experiment involved two separate sets of inoculations because of limited dew chamber space. Fresh inoculum was prepared for each set of inoculations. The first set of inoculated seedlings and controls received 24 hr of dew at two of the test temperatures. The second set of controls and inoculated seedlings received 24 hr of dew at the remaining two test temperatures the following day.

The effect of plant age on disease severity was determined by inoculating seedlings at four different growth stages: 1) cotyledon stage = fully expanded cotyledons, with the first true leaf just beginning to open (10 days); 2) four-leaf stage = four fully expanded true leaves, with the fifth leaf just beginning to expand and the sixth leaf just starting to open (18 days); 3) eight-leaf stage = eight fully expanded true leaves, with the ninth leaf just beginning to open (25 days); and 4) 12-leaf stage = 12–14 fully expanded true leaves with axillary buds present (33 days).

The influence of inoculum concentration on disease severity was determined

**Table 1.** Effect of plant age on disease severity and mortality of tumble pigweed seedlings inoculated with *Aposphaeria amaranthi* after a 24-hr dew period<sup>x</sup>

| Plant age (leaf stage) | Disease severity <sup>y</sup> | Mortality (%) |
|------------------------|-------------------------------|---------------|
| Cotyledon              | 4.2 a <sup>z</sup>            | 79 a          |
| Four leaf              | 5.0 b                         | 100 b         |
| Eight leaf             | 4.2 a                         | 71 a          |
| Twelve leaf            | 1.9 c                         | 6 c           |

<sup>x</sup> Assessments were made 10 days after plants were inoculated with conidial concentrations of  $1-2 \times 10^6$  conidia per milliliter. Dew period and postinfection incubation temperatures were 28 C.

<sup>y</sup> Disease severity rating: 0 = no visible symptoms, 1 = <25% necrosis, 2 = 26–50% necrosis, 3 = 51–75% necrosis, 4 = 76–99% necrosis, 5 = plant death.

<sup>z</sup> Values followed by the same letter in the same column are not significantly different according to LSD ( $P = 0.05$ ).

**Table 2.** Effect of conidial concentration on disease severity and mortality of tumble pigweed seedlings inoculated with *Aposphaeria amaranthi* after a 24-hr dew period<sup>y</sup>

| Concentration (conidia/ml) | Disease severity <sup>z</sup> | Mortality (%) |
|----------------------------|-------------------------------|---------------|
| $10^4$                     | 5.0                           | 100           |
| $10^5$                     | 5.0                           | 100           |
| $10^6$                     | 5.0                           | 100           |
| $10^7$                     | 5.0                           | 100           |

<sup>y</sup> Assessments were made 10 days after plants were inoculated at the four- to six-true-leaf stage. Dew period and postinfection incubation temperatures were 28 C.

<sup>z</sup> Disease severity rating: 0 = no visible symptoms, 1 = <25% necrosis, 2 = 26–50% necrosis, 3 = 51–75% necrosis, 4 = 76–99% necrosis, 5 = plant death.

by spraying plants with conidial suspensions of  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ , and  $1 \times 10^7$  conidia per milliliter.

The effect of dew period duration on disease severity was determined by exposing inoculated seedlings to dew periods of 4, 8, 12, or 24 hr immediately after inoculation. The influence of delayed dew periods on disease severity was determined by spraying plants to runoff with  $2-3 \times 10^6$  conidia per milliliter and maintaining them in the 28 C growth chamber (65% relative humidity). One set of four pots was transferred immediately after inoculation to a 28 C dew chamber. After 12, 24, 48, and 72 hr, sets of four pots were transferred from the growth chamber to the dew chamber for 12 hr.

**Field tests.** Inoculum of *A. amaranthi* was prepared as previously described and adjusted to  $1 \times 10^6$  or  $6 \times 10^6$  conidia per milliliter. Field plots ( $0.5 \times 2$  m) with 1.5-m alleys were established on Nixa cherty silt loam (fragiudults) at the University of Arkansas Agricultural Experiment Station Farm, Fayetteville. Plots were seeded on 5 June 1990 with tumble pigweed and thinned to 40–50 plants per plot after emergence. The test was arranged as a randomized complete block with five replications for each treatment.

Treatments were applied in the evening on 22 June. Plants with two to six true leaves were inoculated at 280, 1,000, and 1,400 (runoff) L/ha for each of the two conidial concentrations. A pump sprayer was used to apply inoculum to runoff. The other applications were made with a CO<sub>2</sub> backpack sprayer at 138 kPa (20 psi); the single boom sprayer was equipped with a flat spray tip nozzle (Teejet 8003, Spraying Systems Co., Wheaton, IL).

Mortality was determined by counting plants the day of inoculation and 1, 2, 3, and 5 wk afterward. Data were subjected to analysis of variance, and treatment means were compared using the

**Table 3.** Effect of dew period on disease severity and mortality of tumble pigweed seedlings inoculated with *Aposphaeria amaranthi*<sup>x</sup>

| Dew period duration (hr) | Disease severity <sup>y</sup> | Mortality (%) |
|--------------------------|-------------------------------|---------------|
| 4                        | 2.7 a <sup>z</sup>            | 30 a          |
| 8                        | 5.0 b                         | 100 b         |
| 12                       | 5.0 b                         | 100 b         |
| 24                       | 5.0 b                         | 100 b         |

<sup>x</sup> Assessments were made 10 days after plants were inoculated at the four- to six-true-leaf stage with  $1-2 \times 10^6$  conidia per milliliter. Dew period and postinfection incubation temperatures were 28 C.

<sup>y</sup> Disease severity rating: 0 = no visible symptoms, 1 = <25% necrosis, 2 = 26–50% necrosis, 3 = 51–75% necrosis, 4 = 76–99% necrosis, 5 = plant death.

<sup>z</sup> Values followed by the same letter in the same column are not significantly different according to LSD ( $P = 0.05$ ).

least significant difference at the 5% significance level.

## RESULTS AND DISCUSSION

**Growth chamber studies.** Plant mortality was not significantly different 2 and 10 days after inoculation, but disease severity on surviving plants was greater after 10 days. The range of dew temperatures conducive for 100% mortality was 20–28 C. Disease severity declined sharply at 32 C, with no plants dying and a mean disease severity of 1.8.

Plant death did not always occur at the cotyledon stage (Table 1). Because seedlings either were killed or had a disease rating of <2, it is possible that seedlings were so small that sufficient inoculum was not maintained on the plants long enough for infection to occur. Other explanations could account for these results, including seedling resistance at the cotyledon stage. Repeat inoculations to surviving plants generally resulted in mortality. Seedlings with four true leaves were consistently killed by *A. amaranthi*. Over 70% mortality was achieved with plants having eight true leaves, but once plants began developing axillary buds, disease severity decreased and symptoms primarily consisted of restricted stem and leaf lesions. These results suggest that *A. amaranthi* may be most effective in controlling tumble pigweed with applications made shortly after emergence (four-leaf stage), since mortality decreases with plant age and also with warmer temperatures.

A potential limiting factor for *A. amaranthi* as a bioherbicide is the inability to produce conidia in liquid culture. Inoculum production in liquid culture is usually considered the most efficient means for mass production (17). However, inoculum production on solid media could be practical if low levels of inoculum are effective. Thus, it is noteworthy that conidial concentrations as low as  $1 \times 10^4$  conidia per milliliter were sufficient to achieve 100% mortality of

**Table 4.** Effect of delaying the onset of a 12-hr dew period on disease severity and mortality of tumble pigweed after inoculation with *Aposphaeria amaranthi*<sup>y</sup>

| Delay (hr) | Disease severity <sup>z</sup> | Mortality (%) |
|------------|-------------------------------|---------------|
| 0          | 5.0                           | 100           |
| 12         | 5.0                           | 100           |
| 24         | 5.0                           | 100           |
| 48         | 5.0                           | 100           |
| 72         | 5.0                           | 100           |

<sup>y</sup> Assessments were made 10 days after plants were inoculated at the four- to six-true-leaf stage with  $2-3 \times 10^6$  conidia per milliliter. Dew period and postinoculation incubation were 28 C.

<sup>z</sup> Disease severity rating: 0 = no visible symptoms, 1 = <25% necrosis, 2 = 26–50% necrosis, 3 = 51–75% necrosis, 4 = 76–99% necrosis, 5 = plant death.

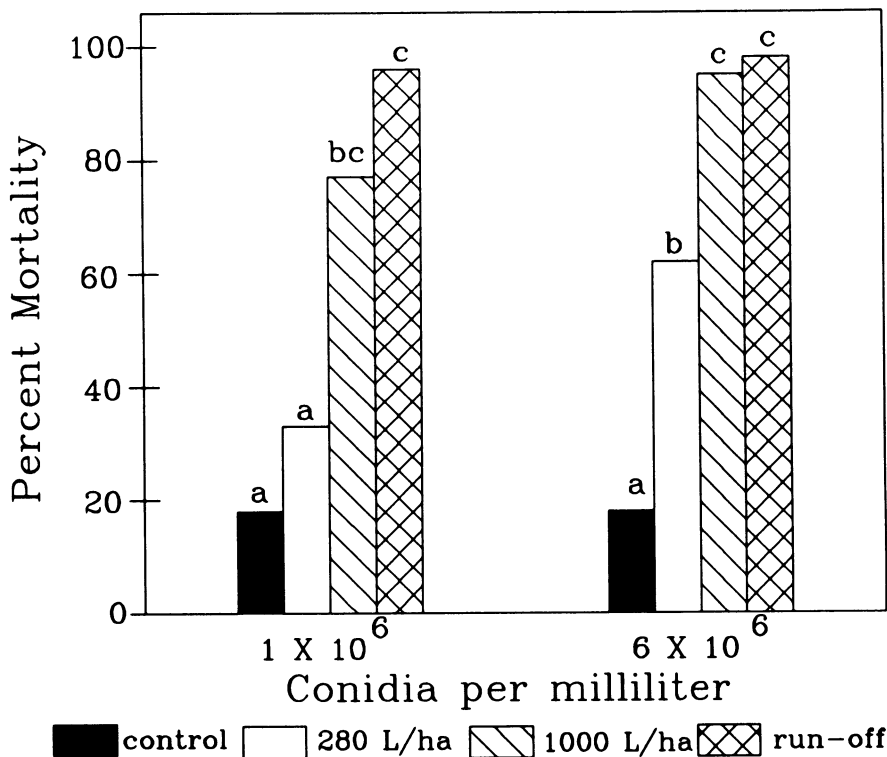


Fig. 1. Comparison of the effects of two conidial concentrations and three application rates of *Aposphaeria amaranthi* on *Amaranthus albus* seedlings in a 1990 field study. Bars labeled with the same letter are not significantly different according to LSD ( $P = 0.05$ ).

tumble pigweed in growth chamber tests (Table 2).

Plant mortality was greatly influenced by dew period. Seedling mortality was 100% after dew periods of at least 8 hr but was reduced to only 30% after a 4-hr dew period (Table 3). The 8-hr dew period requirement is considerably lower than the requirement for many other fungi investigated as potential mycoherbicides (2-4,11,13,14,20,21). In addition to the short dew period requirement, the onset of a 12-hr dew period could be delayed for 3 days without affecting the control levels of tumble pigweed (Table 4).

**Field tests.** A high percentage of tumble pigweed plants always were killed when sprayed to runoff, regardless of concentration (Fig. 1). The highest level of control, 99%, was achieved when seedlings were sprayed to runoff with conidial concentrations of  $6 \times 10^6$  conidia per milliliter, which was not significantly different from plants sprayed to runoff at  $1 \times 10^6$  conidia per milliliter (96% mortality). Differences in the effects of conidial concentrations, however, were significant at the 280 L/ha application rate. The combined effect of conidial concentration and application rate must therefore be taken into account when considering formulation and application techniques.

Growth chamber studies and preliminary field data suggest that *A. amaranthi* has potential for use as a mycoherbicide for tumble pigweed. Preliminary host range tests within the genus have indicated that the fungus is restricted to the Amaranthaceae, but that several species of pigweed, including redroot pigweed, spiny pigweed, and careless weed, also are susceptible to the pathogen, although to a lesser extent than tumble pigweed (12). Further host range tests are needed to evaluate the safety of nontarget plants. More extensive host range tests and screening of additional isolates would also be beneficial in determining the potential of this pathogen as a broad-spectrum mycoherbicide for several different pigweed species. As populations of weeds resistant to chemical herbicides increase and trends toward tillage decrease, alternative methods of controlling pigweed should be considered.

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#### LITERATURE CITED

- Ahrens, W. H., Wax, L. M., and Stoller, E. W. 1981. Identification of triazine-resistant *Amar-*

*anthus* spp. Weed Sci. 29:345-348.

- Cardina, J., Littrell, R. H., and Hanlin, R. T. 1988. Anthracnose of Florida beggarweed (*Desmodium tortuosum*) caused by *Colletotrichum truncatum*. Weed Sci. 36:329-334.
- Cartwright, K. C. 1990. Mycoherbicide potential of a *Colletotrichum* species from pitted morningglory. M.S. thesis. University of Arkansas, Fayetteville. 47 pp.
- Cartwright, R. D., and Templeton, G. E. 1988. Biological limitations of *Protomyces gravidus* as a mycoherbicide for giant ragweed, *Ambrosia trifida*. Plant Dis. 72:580-582.
- Ellis, J. B., and Bartholomew, E. 1896. New Kansas fungi. Erythea 4:1-4.
- Fuerst, E. P., Arntzen, C. J., Pfister, K., and Penner, D. 1986. Herbicide cross-resistance in triazine-resistant biotypes of four species. Weed Sci. 34:344-353.
- Fuerst, E. P., Barrett, M., and Penner, D. 1986. Control of triazine-resistant common lambsquarters (*Chenopodium album*) and two pigweed species (*Amaranthus* spp.) in corn (*Zea mays*). Weed Sci. 34:440-443.
- Holm, L. G., Plucknett, D. L., Pancho, J. V., and Herberger, J. P. 1977. The World's Worst Weeds. University Press of Hawaii, Honolulu. 609 pp.
- LeBaron, H. M., and Gressel, J., eds. 1982. Herbicide Resistance in Plants. John Wiley & Sons, New York. 401 pp.
- Lorenzi, H. J., and Jeffery, L. S. 1987. Weeds of the United States and Their Control. Van Nostrand Reinhold, New York. 355 pp.
- McRae, C. F., and Auld, B. A. 1988. The influence of environmental factors on anthracnose of *Xanthium spinosum*. Phytopathology 78:1182-1186.
- Mintz, A. S. 1991. Evaluation of *Aposphaeria amaranthi* as a potential mycoherbicide for *Amaranthus* spp. M.S. thesis. University of Arkansas, Fayetteville. 99 pp.
- Mortensen, K. 1988. The potential of an endemic fungus, *Colletotrichum gloeosporioides*, for biological control of round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). Weed Sci. 36:473-478.
- Ormeno-Núñez, J., Reeleder, R. D., and Watson, A. K. 1988. A foliar disease of field bindweed (*Convolvulus arvensis*) caused by *Phomopsis convolvulus*. Plant Dis. 72:338-342.
- Ruskin, F. R., ed. 1984. Amaranth. Modern Prospects for an Ancient Crop. Report of an Ad Hoc Panel of the Advisory Committee on Technology Innovation, National Research Council. National Academy Press, Washington, DC. 81 pp.
- Saccardo, P. A. 1899. Supplement 4. Sylloge Fungorum Omnium Hucusque Cognitorum 14:893.
- Templeton, G. E., and Heiny, D. K. 1989. Improvement of fungi to enhance mycoherbicide potential. Pages 127-151 in: Biotechnology of Fungi for Improving Plant Growth. J. M. Whipps and R. D. Lumsden, eds. Cambridge University Press, Cambridge, United Kingdom.
- Vencill, W. K., and Foy, C. L. 1988. Distribution of triazine-resistant smooth pigweed (*Amaranthus hybridus*) and common lambsquarters (*Chenopodium album*) in Virginia. Weed Sci. 36:497-499.
- Weidemann, G. J., TeBeest, D. O., and Cartwright, R. D. 1988. Host specificity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and *C. truncatum* in the Leguminosae. Phytopathology 78:986-990.
- Wymore, L. A., Poirier, C., Watson, A. K., and Gotlieb, A. R. 1988. *Colletotrichum coccodes*, a potential bioherbicide for control of velvetleaf (*Abutilon theophrasti*). Plant Dis. 72:534-538.
- Yang, S.-M., Johnson, D. R., and Dowler, W. M. 1990. Pathogenicity of *Alternaria angustiovoidea* on leafy spurge. Plant Dis. 74:601-604.