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EVALUATION OF *APOSPHAERIA AMARANTHI* AS A BIOHERBICIDE FOR PIGWEED (*AMARANTHUS* SPP.).

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ABSTRACT

Studies were conducted to determine the potential of the fungus, *Aposphaeria amaranthi,* as a bioherbicide for pigweeds (*Amaranthus* spp.). Experiments to establish the environmental parameters necessary for control of tumble pigweed *(A. albus)* demonstrated that an 8-hr dew period was sufficient for control of seedlings with four to six leaves, and that temperatures ranging from 20 to 28 C were conducive for disease development. Conidial concentrations as low as 1 x 10^5 conidia per ml also were sufficient for plant mortality. Host range tests demonstrated pathogenicity of *A. amaranthi* to several other species of *Amaranthus,* including biotypes resistant to triazine herbicides. Disease on redroot pigweed *(A. retroflexus)* was enhanced by incorporation of surfactants into inoculum suspensions. Field tests conducted in1990 resulted in 73% control of redroot pigweed and 99% control of tumble pigweed. These results suggest that *Aposphaeria amaranthi* has potential as a bioherbicide for controlling pigweeds.

INTRODUCTION

Since 1965 more than 250 million acres in the United States have been treated annually with chemical herbicides (Hill, 1982). While chemicals are effective for controlling weeds, their tremendous usage has had undesirable side-effects as well, including residual carry-over (McWhorter and Chandler, 1982), build-up of resistant weed biotypes (Vencill and Foy, 1988), and detrimental effects on the environment. An alternative method for controlling weeds is the use of mycoherbicides, in which fungi are applied inundatively to control or reduce target weed populations (Templeton and Smith, 1977).

The genus *Amaranthus* includes over 60 species, of which the majority are considered weeds, commonly referred to as pigweeds (Ruskin, 1984). Many pigweed species are serious or principal weeds in major crops (Feltner, 1970). Some species have developed biotypes which are genetically resistant to chemical herbicides (Ahrens *et al.*, 1981) and others have been implicated in livestock poisoning, due to high nitrate levels (Holm *et al.*, 1977). In 1987, *Aposphaeria amaranthi* Ell. & Barth., a pycnidial Coelomycete, was isolated from a diseased *Amaranthus* L. species collected at the University of Arkansas Agricultural Experiment Station, Fayetteville. Preliminary host range tests demonstrated pathogenicity of *A. amaranthi* to several *Amaranthus* spp. *Amaranthus albus* L., commonly known as tumble pigweed, was found to be most susceptible. Further studies were conducted to determine the potential of *Aposphaeria amaranthi* as a bioherbicide for pigweed.

MATERIALS AND METHODS

Aposphaeria amaranthi was isolated from symptomatic plant tissues surface disinfested in 1% sodium hypochlorite for 30 sec, rinsed in sterile water for 60 sec, transferred to potato dextrose agar (PDA) (Tuite, 1969) amended with 0.3 mg per ml streptomycin sulfate and incubated at room temperature. Sporulating isolates were stored at -80 °C. Inoculum was prepared by subculturing the fungus on pea juice agar (PJA) (Weidemann *et al.*, 1988) from cultures in cryogenic storage. Cultures were incubated under fluorescent lights (12-hr photoperiod) at 24 to 26 °C for four to six days. Conidia were rinsed from the plates with distilled water and strained through a 1-mm mesh screen. Desired concentrations were standardized using a hemacytometer.

Plants were grown from seed in 28 °C growth chambers (14-hr photoperiod, 330 µE/m2/s). Seedlings were spray inoculated to run-off with conidial suspensions of 1-2 x 10^6 conidia per ml 3 wk after planting, at the four- to six-leaf stage. After the dew period, plants were returned to the 28 °C growth chamber.

Disease severity and plant mortality were determined two and ten days after inoculation. Each treatment consisted of at least three replicated pots with three to five plants. Experiments were repeated at least twice. Controls for each experiment consisted of two pots sprayed with distilled water. Six pots with three to five plants each were used for each species in the host range tests. Assessment of disease severity was based on a rating system of 0 to 5, where 0 = no visible symptoms, 1 = 1-25% necrosis, 2 = 26-50% necrosis, 3 = 51-75% necrosis, 4 = 76-99% necrosis, and 5 = plant death. Inoculated seedlings in the host range tests that showed no visible symptoms were considered immune. Plants that averaged a rating of less than one were considered resistant, and all others were considered susceptible. Data were subjected to analysis of variance and treatment means were compared using the Least Significant Difference at the 5% significance level.

To determine the effect of plant age on disease severity, seedlings were inoculated from the cotyledon stage until axillary buds began to develop. Inoculated seedlings were given a 24-hr dew period at 28 °C. The influence of conidial concentrations was determined by spraying plants with conidial suspensions of 1 x 10^4, 1 x 10^5, 1 x 10^6, and 1 x 10^7 conidia per ml followed by a 12-hr dew period at 28 °C. The dew period requirement was determined by placing inoculated plants in a 28 °C dew chamber and transferring sets of 4 pots to a 28 °C growth chamber after 4, 8, 12, and 24 hr. To determine the effect of dew temperature, inoculated seedlings were given 24-hr dew periods at 20, 24, 28, and 32 °C.

Host range tests included common weed species of *Amaranthus,* as well as triazine-resistant biotypes, and species used as ornamentals and as grain crops. Tests also were conducted on other genera within the Amaranthaceae and on representive genera of related families. Seedlings in the host range tests were given a 24-hr dew period at 28 °C after inoculation with conidial suspensions of *Aposphaeria amaranthi* at 1-2 x 10^6 conidia per ml. Replicated pots of triazine-resistant biotypes of *Amaranthus hybridus* (smooth pigweed) also were sprayed with atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2-4-diamine)], at the recommended field rate of 1.3 mg per 100 ml.

To enhance pathogenicity of *A. amaranthi* on *A. retroflexus* (redroot pigweed), activate plus (Riverside/Terra Corp., Sioux City, IA), agri-dex (Helena Chemical Co., Memphis, TN), soydex (Setre Chemical Co., Memphis, TN), and Mazola corn oil (Best Foods, Inc., Englewood Cliffs, NJ) were incorporated at 0.5% into separate inoculum suspensions of *Aposphaeria amaranthi* at 1-2 x 10^6 conidia per ml and given a 12-hr dew period at 28 °C following inoculations.

Field plots, 0.5 x 2 m, separated by 1.5 m alleys, were established at the University of Arkansas Agricultural Experiment Station, Fayetteville in 1990. Plots were seeded on June 5 with one row of tumble pigweed and one row of redroot pigweed. The test was arranged as a randomized complete block with five replications.

Inoculum for the field study was prepared as previously described and adjusted to 1 x 10^6 conidia per ml and 6 x 10^6 conidia per ml. Treatments were applied on June 22 to plants with two to six leaves. Applications were made at 280 L/ha (30 gpa), 1000 L/ha (100 gpa), and to run-off (1400 L/ha) using a CO_2 backpack sprayer equipped with a single boom flat spray tip nozzle (Teejet 8003) at 20 psi, and with a pump sprayer for plants sprayed to run-off.

RESULTS

Seedlings of tumble pigweed with up to eight leaves were readily killed by *A. amaranthi*. Once plants began developing axillary buds (12 to 14 leaves) disease development decreased and symptoms primarily consisted of restricted stem and leaf lesions. Conidial suspensions of 1 x 10^5 to 1 x 10^7 conidia per ml were sufficient for 100% mortality of tumble pigweed seedlings. When concentrations were decreased to 1 x 10^4 conidia per ml only 75% of the seedlings were killed. Only an 8-hr dew period was necessary for plant death (Table 1), and dew temperatures ranging from 20 to 28 °C were conducive for disease development (Table 2).

Table 1. Effect of dew period on disease severity and mortality of tumble pigweed seedlings 10 days after inoculation with *A. amaranthi* at a concentration of 1-2 x 10^6 conidia/ml at 28 °C.[x]

Dew period duration (hr)	Disease severity[y]	Mortality (%)
4	2.7a	30a
8	5.0b	100b
12	5.0b	100b
24	5.0b	100b

[x]Seedlings (four- to six-leaf stage) were spray inoculated with conidial concentrations of 2 x 10^6 conidia per ml and given dew periods at 28 °C.

[y]Disease severity rating: 0= no visible symptoms, 1= less than 25% necrosis, 2= 26-50% necrosis, 3= 51-75% necrosis, 4= 76-99% necrosis, 5= plant death.

[z]Values followed by the same letter in the same coluan are not significantly different using LSD (P= 0.05).

Table 2. Effect of dew temperature on disease of tumble pigweed seedlings 10 days after inoculation with *A. amaranthi* at a concentration of 1-2 x 10^6 conidia/ml and given a 24 hr dew period.

Dew temperature (C)	Disease severity(y)	Mortality (%)
20	5.0a(z)	100
24	5.0a	100
28	5.0a	100
32	1.8b	0

(y)Disease severity rating: 0= no visible symptoms, 1= less than 25% necrosis, 2= 26-5-% necrosis, 3= 51-75% necrosis, 4= 76-99% necrosis, 5= plant death

(z)Means followed by the same letter in the same column are not significantly different at P= 0.05, according to Duncan's multiple range test

Host range tests demonstrated that with the exception of *Acnidia altissme*, disease incited by *A. amaranthi* was limited to the genus, *Amaranthus*. Plants outside the Amaranthaceae were immune. The majority of *Amaranthus* species, including weeds, ornamentals, and species used as grain crops were susceptible to *Aposphaeria amaranthi*. Biotypes of *Amaranthus* resistant to triazine herbicides also were susceptible.

In growth chamber studies mortality of redroot pigweed seedlings was increased from 33% for plants sprayed with the fungus alone to 93% for plants sprayed with the incorporation of surfactants into inoculum suspensions and given a 12-hr dew period.

Field tests resulted in 73% control of redroot pigweed and 99% control of tumble pigweed when plants were sprayed to run-off with conidial suspensions of 6 x 10^6 conidia per ml. Lower conidial concentrations or application rates were not as effective.

DISCUSSION

Laboratory and field studies demonstrated that *A. amaranthi* is an effective biological control for tumble pigweed. Seedlings with four to six leaves were killed at temperatures ranging from 20 to 28 °C and with conidial concentrations as low as 1 x 10^5 conidia per ml. The dew period requirement necessary for plant death was considerably lower than the dew period required by most fungi investigated as potential bioherbicides. Applications made shortly after emergence probably would be most effective since mortality decreases with plant age and with temperatures above 28 °C.

Effective control levels of redroot pigweed were achieved in field tests only with a combination of high conidial concentrations and high application rates. Growth chamber studies, however, indicated that mortality of redroot pigweed could be increased with incorporation of surfactants into inoculum suspensions. Results from host range tests suggest that *A. amaranthi* is restricted to the Amaranthaceae and would pose little threat to non-target plants. These results suggest that *Aposphaeria amaranthi* has potential as a bioherbicide for pigweed.

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