Interaction of *Alternaria macrospora* and *Fusarium lateritium* on Spurred Anoda

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ABSTRACT

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The interaction of diseases incited by *Alternaria macrospora* and *Fusarium lateritium* on spurred anoda (*Anoda cristata*) plants was influenced by levels of inocula, ratios of inocula, and the sequence of inoculation for each pathogen. The highest levels of plant mortality were obtained when *A. macrospora* was applied 5 days before *F. lateritium*, and the lowest levels of plant mortality were obtained when *F. lateritium* was applied 5 days before *A. macrospora*. This interaction is potentially useful to increase the effectiveness of the two pathogens as mycoherbicides.

Spurred anoda (*Anoda cristata* (L.) Schlecht.) (Malvaceae) is an economically important weed in much of the cotton-producing areas of the United States (5,6). This weed is both difficult and expensive to control with conventional herbicides, and there is a need for the development of better control methods (5,6).

An indigenous fungus, *Alternaria macrospora* Zimm., has been investigated as a mycoherbicide for spurred anoda (8,10–12,15,16). This isolate of *A. macrospora* was shown to be a specialized form of the fungus that was restricted in host range (15,16). Methods were developed to produce conidia for foliar treatments (10), and granular formulations of mycelium were also developed for preemergence or postemergence applications in the field (11,14). *A. macrospora* controlled spurred anoda seedlings best when inoculations were made during the cotyledon to first-leaf growth stage (12). Plants larger than the fourth-leaf stage were not as severely damaged as younger plants. Greenhouse tests indicated that the optimum dew periods for killing spurred anoda seedlings were 24 hr or longer at 20–30 C. Inoculum concentrations of 2.5 to 10 × 10^5 conidia per milliliter produced the best control. In field tests, foliar (12) and granular (11) formulations of the pathogen significantly reduced the total dry weights of spurred anoda by 68 and 72%, respectively.

During an epiphytotic, *Fusarium lateritium* Nees ex Fr. and *A. macrospora* were isolated from the same stem lesions of severely diseased spurred anoda plants (13). Many plants 1 m or taller were killed. This isolate of *F. lateritium* also infected prickly sida (*Sida spinosa* L.), velvetleaf (*Abutilon theophrasti* Medic.), Venice mallow (*Hibiscus trionum* L.), okra (*Abelmoschus esculentus* (L.) Moench), and hollyhock (*Althaea rosea* (L.) Cav.). No disease symptoms were produced on corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L. and *G. barbadense* L.), soybean (*Glycine max* (L.) Merr.), and 18 other crop and weed species representing eight families (13).

This study was initiated to evaluate combinations of *A. macrospora* and *F. lateritium* as mycoherbicides for spurred anoda because preliminary results indicated that neither *A. macrospora* (12) nor *F. lateritium* (H. L. Walker, *unpublished*) severely damaged spurred anoda seedlings larger than the fourth-leaf growth stage unless the plants were wound-inoculated.

Two host-specific pathogens have been combined to control two weed species (2), and individual pathogens have been proposed as mycoherbicides for several weeds (13), but enhancement of the effectiveness of biological control agents by the combined use of two pathogens for one weed species has not been reported.

MATERIALS AND METHODS

The *F. lateritium* and *A. macrospora* used in these studies were isolated from diseased spurred anoda plants collected near Stoneville, MS (13).

About 50 g of *A. macrospora* conidia were produced by previously described methods (10) and stored at 4 C until needed. These conidia were the source of *A. macrospora* inoculum for all tests. One-tenth of a gram of spores suspended in 1 L of water contained about 1 × 10^5 conidia per milliliter as determined with a hemacytometer.

Macroconidia of *F. lateritium* were produced as previously described (13) in disposable petri dishes (15 × 100 mm) of vegetable juice agar (7) supplemented with L-proline (1.3 g/L). A sterile distilled water rinse was used to harvest the macroconidia from 7- to 10-day-old cultures as inoculum was needed. An average of 2.7 × 10^7 macroconidia was harvested from each petri dish culture.

The plants were greenhouse-grown, as described previously (13), from acid-scarified seeds in 5-cm-square peat pots. The potting mix (George J. Ball Company, West Chicago, IL) was supplemented with a 14-14-14 (NPK) slow-release fertilizer. Groups of 12 plants in the fourth- to fifth-leaf stage were used as experimental units.

Experiments were divided into three series of tests. Series one (*A. macrospora*/*F. lateritium*) consisted of *A. macrospora* applied 5 days before *F. lateritium*, series two (*F. lateritium*/*A. macrospora*) consisted of *F. lateritium* applied 5 days before *A. macrospora*, and series three (*A. macrospora* + *F. lateritium*) consisted of both pathogens applied simultaneously. The 5-day interval, as determined by preliminary testing, allowed sufficient time for the pathogen applied first to establish infection sites before the second pathogen was applied.

Each pathogen was applied at inoculum levels of 0, 5 × 10^3, 5 × 10^4, and 1 × 10^5 spores per milliliter. Thus, each series of tests consisted of 16 treatments and 768 plants. The spray mixtures consisted of conidia, 0.05% (w/v) Tween 80, and distilled water. Plants were sprayed to runoff with an atomizer and placed in dew chambers (Percival model I-35D, Percival Manufacturing Company, Boone, IA) for 18–21 hr at 25 C, then moved to greenhouse benches.

The number of plants killed was determined at 5-day intervals up to 30 days after application of inocula. Each treatment was replicated four times in a randomized complete block experimental design with a factorial arrangement of treatment combinations for each series. All experiments were conducted twice, and the data presented are the averages

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for both experiments. Significant differences among mean values for each treatment were determined at $P = 0.05$ with Fisher's LSD (9).

RESULTS AND DISCUSSION

Spurred anoda injury was influenced by concentrations of inocula and sequence of application of inocula. The highest rate of kill (100%) was obtained with the *A. macrospora*/*F. lateritium* sequence of application (Fig. 1), *A. macrospora* + *F. lateritium* was intermediate (79%) (Fig. 2), and the lowest rate of kill (11%) was obtained with the *F. lateritium*/*A. macrospora* sequence of application (Fig. 3).

Although an average of 79% of the plants were killed as a result of the *A. macrospora* + *F. lateritium* sequence of application, there was a 5- to 10-day lag in disease development compared with the results of the *A. macrospora*/*F. lateritium* series (Fig. 4). When *F. lateritium* was applied 5 days before *A. macrospora*, no significant enhancement of disease severity occurred (Fig. 3).

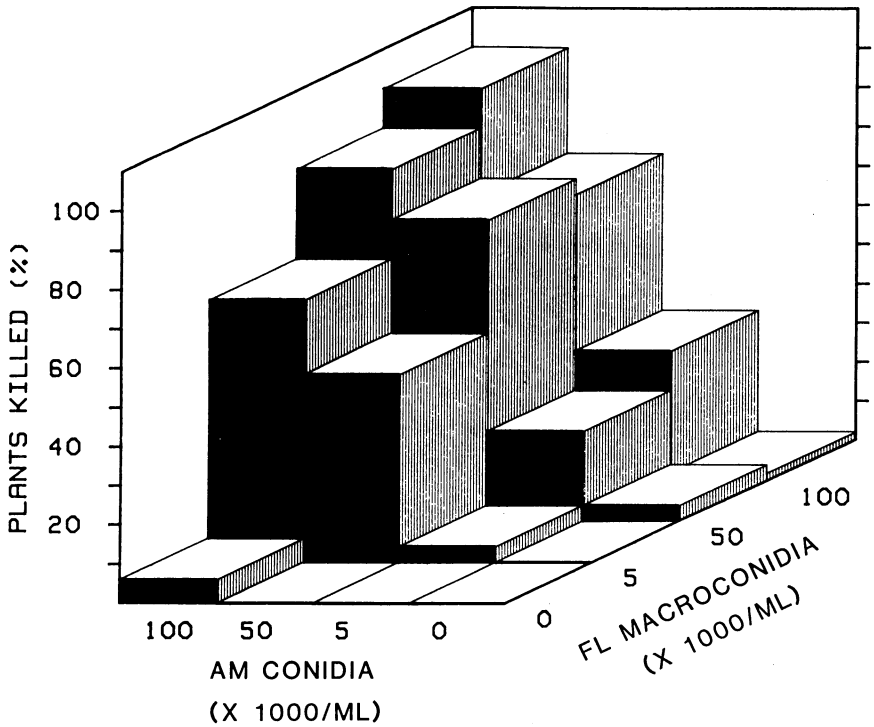

Fig. 1. Mortality of spurred anoda plants 30 days after applying inoculum of *Alternaria macrospora* (AM) and 25 days after spray inoculating the same plants with inoculum of *Fusarium lateritium* (FL). LSD (0.05) = 11.4%.

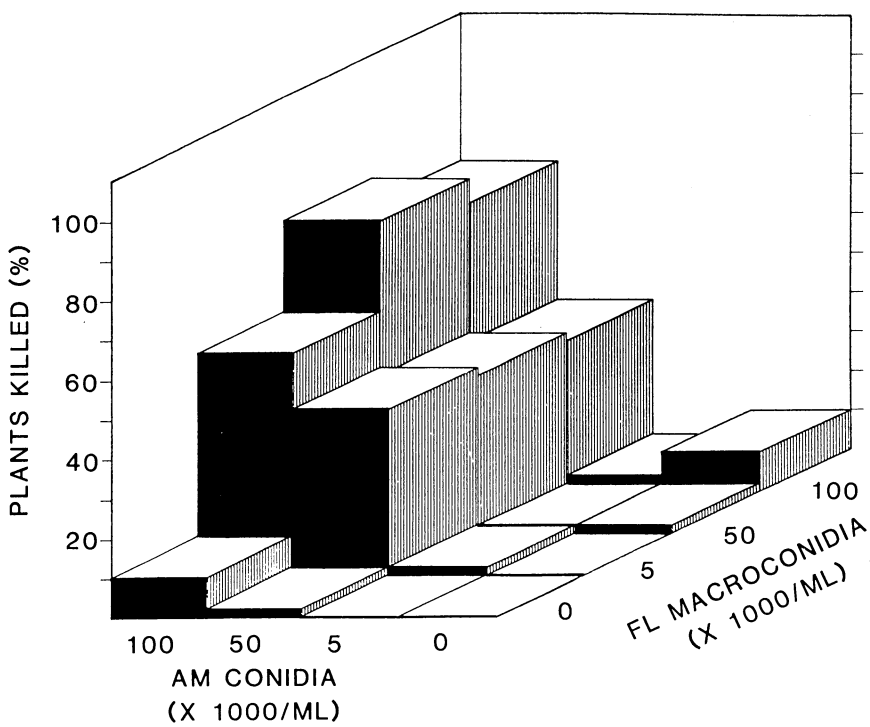

Fig. 2. Mortality of spurred anoda plants 30 days after simultaneous application of inoculum of *Alternaria macrospora* (AM) and *Fusarium lateritium* (FL). LSD (0.05) = 15.6%.

Spurred anoda plants must be controlled within 4–6 wk after cotton is planted to prevent yield losses (5,6). Thus, any effective mycoherbicide must produce relatively rapid control of young spurred anoda seedlings in row-crop environments characterized by intensive cultivation and heavy pesticide use.

The synergistic interaction between these two pathogens could be used to make both pathogens more effective as mycoherbicides. Spurred anoda seedlings increase in tolerance to each of these pathogens with each successive growth stage (12,13). The *A. macrospora*/*F. lateritium* and *A. macrospora* + *F. lateritium* combinations used in these studies usually killed 100% of spurred anoda seedlings that were inoculated in the cotyledon to first-leaf stage, so it was necessary to use plants in the fourth- to fifth-leaf stage to demonstrate the effects of concentration of inocula and ratios of inocula on the synergistic interaction.

Although *A. macrospora* readily infected older plants after foliar applications, injury was usually limited to necrotic leaf spots and superficial stem lesions. *A. macrospora* killed only 10% of these older plants, even when wound-inoculated. In contrast, foliar applications of *F. lateritium* produced only a limited number of infection sites, yet plants were usually killed when *F. lateritium* was wound-inoculated, regardless of the size of the plants at the time of inoculation. Thus, one explanation for the *A. macrospora*/*F. lateritium* interaction is that *F. lateritium* penetrated and infected through the *A. macrospora* infection sites.

When agar plugs of *A. macrospora* and *F. lateritium* were placed in the same petri dishes of growth medium, zones of inhibition were evident as *F. lateritium* inhibited the growth of *A. macrospora*. This inhibition is consistent with previously reported research. *F. lateritium* is known to occur as a saprophyte or as a weak parasite and to produce enniatins, antifungal compounds (1). Isolates of *F. lateritium* have been used to protect wounded apricot (*Prunus* sp.) against the vascular pathogen *Eutypa armeniacea* Hansf. & Carter (3,4).

This inhibition of *A. macrospora* by *F. lateritium* could account for the decreased disease severity that resulted when *F. lateritium* was applied prior to or simultaneously with *A. macrospora*. These results indicate that once infection sites were established by *A. macrospora*, as in the *A. macrospora*/*F. lateritium* inoculation sequence, this inhibition

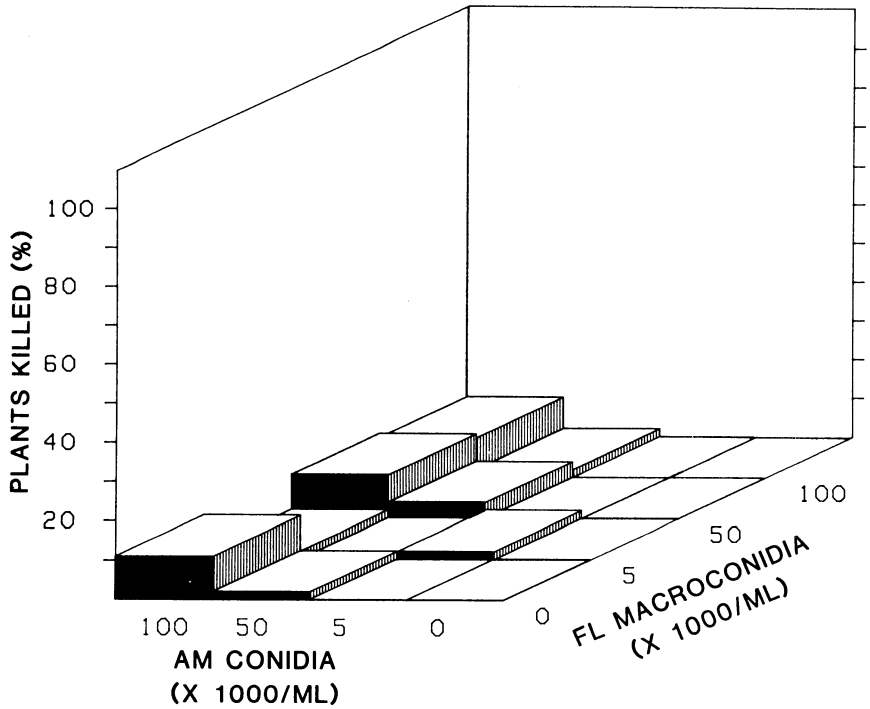

Fig. 3. Mortality of spurred anoda plants 30 days after applying inoculum of *Fusarium lateritium* (FL) and 25 days after spraying the same plants with inoculum of *Alternaria macrospora* (AM). LSD (0.05) = 7.0%.

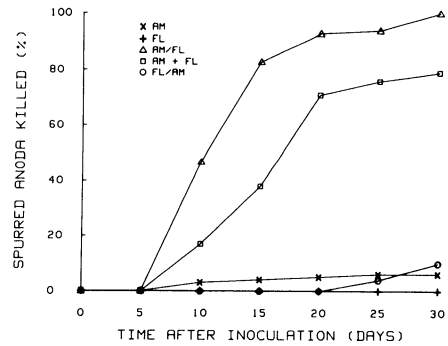

Fig. 4. Mortality of spurred anoda plants after various combinations of *Alternaria macrospora* (AM) and *Fusarium lateritium* (FL). Inoculum levels for each pathogen were 1×10^5 spores per milliliter. AM/FL = *A. macrospora* applied 5 days before *F. lateritium*, AM + FL = *A. macrospora* and *F. lateritium* applied simultaneously, FL/AM = *F. lateritium* applied 5 days before *A. macrospora*.

became less important as a factor in disease development.

These results indicate that combinations of *A. macrospora* and *F. lateritium* are more effective than either pathogen used alone for control of spurred anoda. Whereas simultaneous applications of the pathogens would be more practical, for maximum effectiveness, the results reported here indicate that *A. macrospora* should be applied 5 days before *F. lateritium*.

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