Influence of Sequential Dew Periods on Biocontrol of Sicklepod (*Cassia obtusifolia*) by *Alternaria cassiae*

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ABSTRACT

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In greenhouse tests, at least 8 hr of dew within 2 days after inoculation was required for the fungus *Alternaria cassiae* to kill all inoculated sicklepod seedlings consistently. Increased efficacy of *A. cassiae* was evident when inoculated seedlings were treated with two shorter than optimal dew periods of 2–6 hr 1, 2, 3, or 4 days after the initial dew period. For *A. cassiae* to be used as a microbial herbicide, inoculum should be applied when environmental conditions are favorable for dew to occur on the foliage of sicklepod within 2 days after inoculation.

Sicklepod (*Cassia obtusifolia* L.) is a problem weed in many fields of soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.), and peanut (*Arachis hypogaea* L.) in the southeastern United States (3,5–7,9,11). Seedlings emerge throughout the growing season under a wide range of environmental conditions (11). Sicklepod is resistant to many herbicides (4), and a combination of weed management practices is often necessary for effective control (6).

The fungus *Alternaria cassiae* Jurair & Khan has been evaluated as a microbial herbicide to control sicklepod (12,15,17). A dew period of at least 8 hr is required for conidia of *A. cassiae* to germinate and provide 100% control of sicklepod seedlings. No attempts were made to determine the effects of delayed or multiple dew periods on infection and kill of sicklepod. Under field conditions, dew formation and duration can be difficult to predict; often, several days will pass without any measurable leaf wetness. More information is needed on the effects of dew duration to understand the environmental conditions necessary for this mycoherbicide to control sicklepod better. The study described in this paper was initiated to determine the effects of two shorter than optimal dew periods and the effects of delaying either the initial or the second dew for 1, 2, 3, or 4 days.

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MATERIALS AND METHODS

The isolate of *A. cassiae* used in these tests was the same as that studied previously (17). Culture conditions and methods for production and quantitation of inoculum were described (17). About 200 g of a conidial preparation (1×10^8 conidia per gram) was produced and stored at 4 C. These conidia were used as inoculum for all tests.

Sicklepod seedlings were grown in a greenhouse from acid-scarified seeds planted in strips of 12 peat pots (5.5 cm square) (Jiffy Products [NB] Ltd., Shippegan, Canada). The potting medium and greenhouse conditions have been described previously (17). Seedlings were inoculated in the cotyledon- to first-leaf stage by spraying until runoff with aqueous preparations containing 1×10^5 conidia per milliliter and 0.02% (v/v) nonoxynol (9–10 POE) [α-(*p*-nonylphenyl)-ω-hydroxypoly(oxyethylene)]surfactant. Control plants were sprayed with water containing 0.02% surfactant only.

Immediately after inoculation, the plants were placed on greenhouse benches (0 hr dew) or in dew chambers (25 C) for 2, 4, 6, or 8 hr, then moved to greenhouse benches. A second dew period of 0, 2, 4, 6, or 8 hr followed 1, 2, 3, of 4 days after the initial dew period. The percentage of plant mortality was determined 2 wk after inoculations.

All 100 treatments were replicated three times with 12 plants per experimental unit. Each experiment was repeated twice, using a randomized complete block design with a factorial arrangement of treatments (10).

RESULTS AND DISCUSSION

A single dew period at least 6–8 hr was needed for 90–100% kill of sicklepod seedlings. The maximum rate of kill was obtained with the shortest delay in the occurrence of dew from the time of inoculation. When an initial 8-hr dew period was delayed up to 2 days after inoculation, rate of kill in sicklepod was equal to plants receiving dew periods immediately after inoculation (Fig. 1). When the initial dew period was delayed 3 days or longer after inoculation, however, there were significant reductions in the numbers of plants killed.

Two successive dew periods of 4–8 hr enhanced weed kill (Figs. 2–5). The second dew periods produced the greatest increase in seedling mortality when they occurred within 2 days after the first dew. The results from successive dew periods were not additive, however. For example, two successive 4-hr dew periods produced less seedling mortality than did one 8-hr dew period.

The initial dew period had a greater effect on disease development than the second dew period. For example, successive dew periods of 6 and 4 hr resulted in consistently higher mortality than did successive dew periods of 4 and 6 hr (Figs. 2–5).

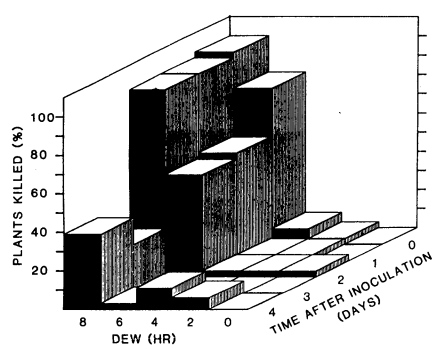

Fig. 1. Effect of delaying initial dew periods on kill of sicklepod plants by *Alternaria cassiae*. The relationship was best described ($P = 0.0001$, $r^2 = 0.88$) by the following equation: $Y = 4.65 + 3.18D + 1.80D^2\ 2.63T$, where D = length of dew period (hr) and T = time after inoculation (days).

The optimum dew periods for maximum disease development can vary widely with respect to species or isolates (1,2,13,14). The optimum dew period for *A. cucumerina* (Ellis & Everh.) Elliot was 24 hr at 20 C (13), whereas *A. helianthi* (Hansf.) Tubaki & Nishihara produced maximum levels of infection with a 12-hr dew period at 25 C. Lesion size of *A. helianthi* on sunflower (*Helianthus annuus* L.) was significantly increased with 10 sequential dew periods of 10–15 hr each compared with plants treated with two dew periods 10–15 hr each (1). In other studies, an isolate of *A. macrospora* Zimm. pathogenic to cotton (*G. barbadense* L.) produced maximum weed control after a 9-hr dew period at 25 C (2), whereas another isolate of *A. macrospora* pathogenic to spurred anoda (*Anoda cristata* (L.) Schlecht.) required dew periods of >24 hr at 25 C (14).

Any commercially successful mycoherbicide must produce a consistent, predictable level of weed control when applied to the target weed according to established protocols. An understanding of the effects of varying dew periods on sicklepod infection by *A. cassiae* will help determine the optimum timing of applications and the most suitable formulations to provide a consistent level of weed control. *A. cassiae* would be most effective as a mycoherbicide if applications were made when conditions favor the occurrence of at least 8 hr of dew on inoculated sicklepod plants. A delay of 1 or 2 days in the occurrence of dew might be tolerated without adversely affecting the efficacy of this pathogen. On the basis of these results, successive dew periods shorter than 8 hr each would be conducive to disease development when they occur within 2 days after inoculation.

Leaves of sicklepod plants fold as a result of phototropic response to low light intensity; this phenomenon makes herbicide application and deposition difficult (8). The results of these tests indicate that conidia of *A. cassiae* can tolerate repeated periods of wetting and drying. Therefore, inoculum applications can be made throughout the day and eliminate the need for late afternoon or evening applications. Thus the phototropic response phenomenon exhibited by sicklepod would not seriously interfere with biological control efforts.

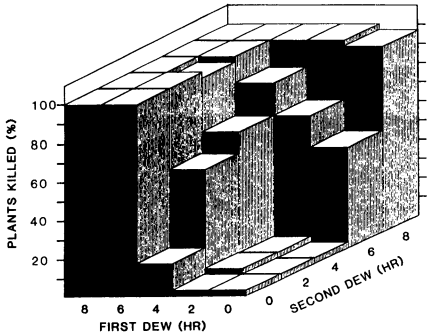

Fig. 2. Effects of two sequential dew periods of various durations on kill of sicklepod plants by *Alternaria cassiae*. The first dew periods were initiated immediately after inoculation and the second dew periods were initiated 1 day later. The relationship was best described ($P = 0.0001$, $r^2 = 0.83$) by the following equation: $Y = 21.70 + 9.97F - 7.35F^2 1.75S$, where Y = percent sicklepod killed, F = length of first dew (hr), and S = length of second dew (hr). The combined results of two repeats in time are presented.

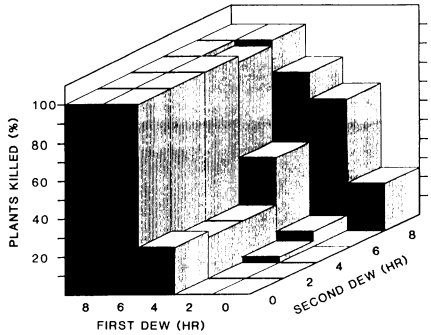

Fig. 4. Effects of two sequential dew periods of various durations on kill of sicklepod plants by *Alternaria cassiae*. The first dew periods were initiated immediately after inoculation and the second dew periods were initiated 3 days later. The relationship was best described ($P = 0.0001$, $r^2 = 0.84$) by the following equation: $Y = -12.33 + 14.09F + 0.99F^2 - 0.62S$, where Y = percent sicklepod killed, F = length of first dew (hr), and S = length of second dew (hr). The combined results of two repeats in time are presented.

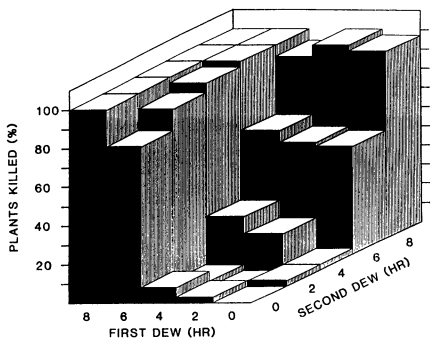

Fig. 3. Effects of two sequential dew periods of various durations on kill of sicklepod plants by *Alternaria cassiae*. The first dew periods were initiated immediately after inoculation and the second dew periods were initiated 2 days later. The relationship was best described ($P = 0.0001$, $r^2 = 0.80$) by the following equation: $Y = 23.70 + 25.83F + 0.75F^2 - 1.88S$, where Y = percent sicklepod killed, F = length of first dew (hr), and S = length of second dew (hr). The combined results of two repeats in time are presented.

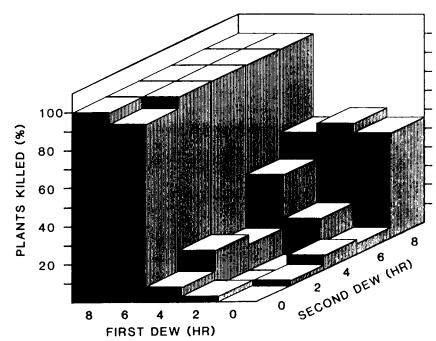

Fig. 5. Effects of two sequential dew periods of various durations on kill of sicklepod plants by *Alternaria cassiae*. The first dew periods were initiated immediately after inoculation and the second dew periods were initiated 4 days later. The relationship was best described ($P = 0.0001$, $r^2 = 0.71$) by the following equation: $Y = -13.8 + 9.00F + 1.78F^2 - 0.88S$, where Y = percent sicklepod killed, F = length of first dew (hr), and S = length of second dew (hr). The combined results of two repeats in time are presented.

ACKNOWLEDGMENT

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