Temperature and Moisture Requirements for Development of Anthracnose on Northern Jointvetch

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ABSTRACT

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Colletotrichum gloeosporioides f. sp. *aeschynomene* is the causal agent of anthracnose on northern jointvetch. In controlled environments, disease developed rapidly between 20 and 32 C, but development was most rapid at 28 C. At 28 C, 16- to 18-day-old seedlings died within 8 days after inoculation. Incubation of inoculated seedlings in alternating day/night temperature regimes of 32/24 C or 28/20 C reduced the rate of disease development compared with that at 28 C. Dew periods of at least 12 hr at 28 C were required to obtain infection of all seedlings. Incubation of seedlings at 24 or 32 C during the dew period increased the time required for disease development, and temperatures of 20 and 36 C during the dew period further reduced disease severity. Temperatures and dew periods in the rice field environment are similar to those required for rapid growth and development of the organism on northern jointvetch in the laboratory.

Additional key words: mycoherbicide, biological weed control, weed control, herbicide, rice.

Northern jointvetch, *Aeschynomene virginica* (L.) B.S.P., is a troublesome weed in rice fields of Arkansas, Mississippi, and Louisiana (11). *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* (nomen nudum) incites an anthracnose disease on northern jointvetch. Considerable interest has developed concerning its potential use as a mycoherbicide (1). Evidence that this fungus is effective for weed control in rice fields is a prerequisite for its registration and use as a mycoherbicide. The purpose of this research was to determine environmental requirements for development of anthracnose on northern jointvetch.

MATERIALS AND METHODS

General.—Seedlings of northern jointvetch were grown from seed collected from plants harvested near Stuttgart, Arkansas. Seeds were treated with 0.5% NaOCl, scarified, again treated with the NaOCl solution, and plated on moist filter paper disks in petri dishes and kept for 24 hr at 28-32 C. After 24 hr, germinated seeds were planted 0.5 cm deep in pasteurized field soil in 7.1-cm diameter plastic pots. All pots then were placed in a controlled-environment growth chamber (28 C, 7,642 lux, 40-100% RH, 15-hr day-length). Seedlings were inoculated when they were 15- to 18-cm-tall (16 to 18 days old) in all experiments. After inoculation, the seedlings were transferred to controlled-environment growth chambers (1,991 lux, 40 to 100% RH, and a 15-hr day-length) under different temperature regimes.

The inoculum used in all experiments was obtained from 3- to 4-day-old liquid cultures of *C. gloeosporioides* f. sp. *aeschynomene* that had been grown in 50 ml of modified Richard's solution fortified with V-8 juice in 125-ml Erlenmeyer flasks shaken at 250 rpm at 28 C (1). Spores were collected by filtration of culture fluid through filter paper (Sargent-Welch 9-975C). Spores in the filtrate were pelleted, washed three times by centrifugation (1,050 *g*) and resuspended in distilled water. The suspensions were diluted to a final concentration of 10^6 spores/ml by comparison with an absorption curve previously established with a Bausch and Lomb Spectronic 20 spectrophotometer set at 525 nm. The inoculum was diluted to lower concentrations in several experiments. All plants were inoculated with spore suspensions by application of aerosol sprays until droplets formed on plant stems.

A disease index based on lesion type, lesion size, and overall plant condition was developed to describe the severity of the disease on individual plants. Numerical values for this index were: 0, no lesions; 1, pinpoint lesions of 0.5-1.0 mm diameter; 2, lesions up to 1 cm diameter but not encircling the stem; 3, lesions longer than 1 cm or encircling the stem; 4, plant above lesions flaccid or collapsed; and 5, plant dead.

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All experiments were replicated three times; each treatment in a replicate contained 10 seedlings.

All data were analyzed statistically and means were separated by Duncan's multiple range test for significance, $P = 0.05$. Percentage values were arc-sin transformed before analysis.

Effect of spore concentration. Seedlings were inoculated with spore suspensions containing 10^6, 10^5, 10^4, 10^3, or 10^2 spores/ml. Inoculated seedlings were placed in a dew chamber at 28 C for 24 hr, and then were returned to a growth chamber at 28 C. Disease development was recorded as the number of lesions per centimeter of stem on all inoculated plants 3 days after inoculation and the percentage of plants infected 10 days after inoculation. The lesions were counted 3 days after inoculation on 2 cm of stem randomly selected between the first and second leaves of each plant.

Effect of dew period.—Seedlings were placed in a dew chamber at 28 C for 4, 8, 12, or 24 hr after inoculation and then returned to the growth chamber at 28 C. Plants that received no dew-period treatment were placed in the growth chamber immediately after inoculation. Droplets of the inoculum on the stems and leaflets dried within 15 min after the plants were placed in the growth chamber. Disease development was recorded as the percentage of plants infected and the disease index values of infected plants 5 and 10 days after inoculation.

TABLE 1. Effect of inoculum density on severity of an anthracnose disease caused by *Colletotrichum gloesporioides* f. sp. *aeschynomene* on northern jointvetch[x]

Inoculum spore concentration (no./ml.)	Plants infected[y] (%)	Lesions on stem[z] (no./cm)
10^2	30 a	0.0 a
10^3	60 ab	0.05 a
10^4	93 b	0.1 b
10^5	100 b	0.9 c
10^6	100 b	4.0 d

[x]In each column means followed by the same letter are not significantly different by Duncan's multiple range test ($P=0.05$).
[y]Ten days after inoculation.
[z]Lesions were counted 3 days after inoculation on 2 cm of stem randomly selected between the first and second leaves of all plants.

TABLE 2. Effect of the length of the postinoculation dew period on the severity of anthracnose on northern jointvetch inoculated with 10^6 spores/ml

Dew period (hr)	Plants infected (%) at 5 days	Plants infected (%) at 10 days	Disease index of infected plants at 5 days	Disease index of infected plants at 10 days
0	0.0 a[z]	0.0 a	0.0 a	0.0 a
4	3.3 a	10.0 b	2.0 a	2.7 b
8	33.3 b	50.0 c	2.2 ab	4.3 c
12	96.7 c	100.0 d	4.2 b	4.9 c
24	100.0 c	100.0 d	4.5 b	5.0 c

[z]Numbers in each column followed by the same letter are not significantly different by Duncan's multiple range test ($P=0.05$).

Effect of temperature during dew period.—Seedlings were placed in a dew chamber for 12 hr at 20, 24, 28, 32, or 36 C after inoculation and then returned to a growth chamber at 28 C. Disease development was recorded as the percentage of plants infected at 5 and 10 days after inoculation and as daily disease index values at 1-10 days after inoculation.

Effect of air temperature during incubation period.—Following inoculation, seedlings were incubated for 24 hr at 28 C in a dew chamber and then placed in growth chambers with either constant air temperature of 16, 20, 24, 28, or 32 C, or with a day/night temperature regime of 32/24 C or 28/20 C. Disease development was measured as daily disease index values at 1-10 days after inoculation.

RESULTS

Symptom development.—Several stages were identified in the development of the stem lesions. Initial symptoms of the disease were pinpoint lesions, 0.5 to −1.0 mm in diameter, that developed within 48 hr after inoculation. Lesions rapidly enlarged until the stems were girdled. Once the stems were girdled, plant parts above the girdling lesions collapsed and died.

Effect of spore concentration.—The percentage of inoculated plants that were infected was influenced by inoculum density (Table 1). When inoculated with 10^5 or 10^6 spores/ml, all seedlings became infected and died within 7 to 9 days. The percentage of infected plants was significantly lower when the inoculum contained 10^2 spores/ml. The number of lesions that developed on the stem also was related directly to the concentration of the inoculum. No lesions were counted on randomly selected stem segments of plants treated with 10^2 spores/ml. The number of lesions increased as the inoculum concentration was increased from 10^3 to 10^6 spores/ml. A concentration of 10^5 spores/ml resulted in infection of 100% of the plants or only 7% more than were infected by 10^4 spores/ml. However, plants inoculated with 10^5 spores/ml had nine times more stem lesions/cm and died 2 days sooner than those inoculated with 10^4 spores/ml. Similarly, plants inoculated with 10^6 spores/ml had more than four times more stem lesions/cm and died 2 days earlier than those inoculated with 10^5 spores/ml. Lesions coalesced, girdled stems, and killed weed seedlings more quickly at the higher, than at the lower, spore concentrations.

Effect of dew period.—Free moisture or dew was required for infection of weed seedlings (Table 2). Symptoms were evident within 10 days on plants that had been incubated either for 12 or 24 hr in a dew chamber at 28 C; however, a 24-hr dew period resulted in more rapid symptom development. As the dew period was decreased to less than 12 hr, fewer seedlings were infected; at least 12 hr of free moisture were required to obtain 100% infection at 28 C.

Disease also developed more slowly with a dew period of 4 hr than with one of 8 hr. Plants were severely diseased within 10 days when the dew period was 8 hr or more. When the dew period was 8 hr, lesions remained relatively small (1 cm or less) after 5 days, but after 10 days some dead seedlings were observed. Even after 10 days,

however, lesions remained small when inoculated plants had received only 4 hr of dew.

Effect of temperature during dew period.—Incubation temperature during the dew period influenced the number of plants infected, and the rate of disease development (Fig. 1). When inoculated plants were incubated in a dew chamber for 12 hr at 24, 28, or 32 C, plants became infected and the disease developed rapidly. Although the disease developed most rapidly at 28 C, disease-index values 10 days after inoculation did not differ significantly ($P > 0.05$) for incubation temperatures of 24, 28, and 32 C. However, dew-period temperatures of 20 and 36 C significantly ($P > 0.05$) reduced disease-index values relative to those of the other three dew-period-temperature treatments. At 36 C, only one plant of 30 inoculated became visibly infected during the 10-day period. With a dew-period temperature of 20 C, 30% of the plants were infected, but only one plant died after 10 days of incubation.

Effect of air temperature during incubation period.—Air temperatures in growth chambers affected the rate of disease development (Fig. 2 and 3). In constant-temperature regimes disease developed most rapidly at 28 C (Fig. 2); however, at 5 days after inoculation, disease-index values did not differ significantly ($P > 0.05$) for temperatures of 20, 24, 28, or 32 C. At constant temperatures of 20 to 32 C, pinpoint lesions were evident within 2 or 3 days and expanded rapidly to kill plants 5-8 days after inoculation. At a constant temperature of 16 C, the rate of disease development and the severity of the disease were significantly ($P > 0.05$) reduced 5 and 10 days after inoculation; pinpoint lesions were not visible until 4 days after inoculation, and the average disease-index value was 3.9 at 10 days after inoculation.

Compared to a constant temperature of 28 C, alternating temperature regimes inhibited disease development (Fig. 3). A day/night temperature regime of 32/24 C significantly ($P > 0.05$) reduced disease-index values at 5-10 days after inoculation. The rate of disease development was similar in the two alternating temperature regimes until 6 days after inoculation when lesions appeared to expand at different rates. Thereafter, disease-index values were significantly ($P > 0.05$) lower with the 32/24 C day/night temperature regime than with the 28/20 C regime.

DISCUSSION

Daniel et al. (1) reported that *C. gloeosporioides* f. sp. *aeschynomene* satisfies the requirements of a biological

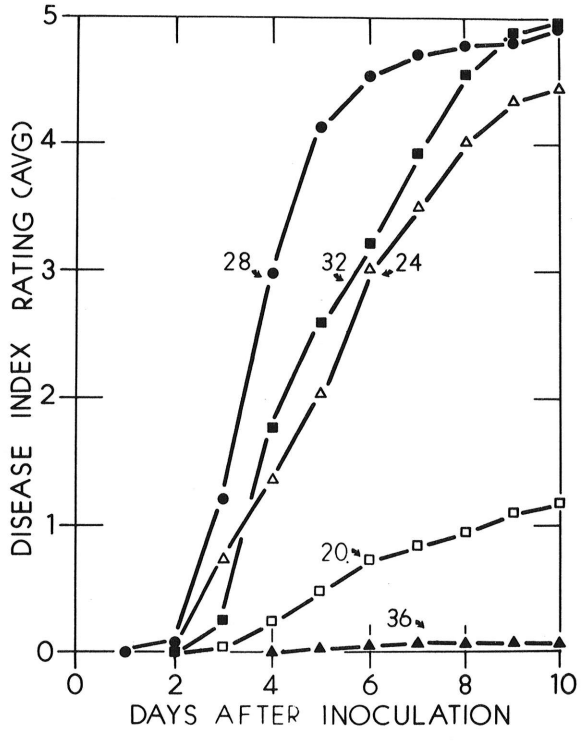

Fig. 1. Effect of temperature during the dew period on development of anthracnose (caused by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*) in northern jointvetch. Plants were inoculated with 10^6 spores/ml, incubated for 12 hr in a dew chamber at the indicated temperatures, and then moved to a growth chamber held at a constant temperature of 28 C. Disease index ratings are averages of three replications where 0 = no infection and 5 = plant death.

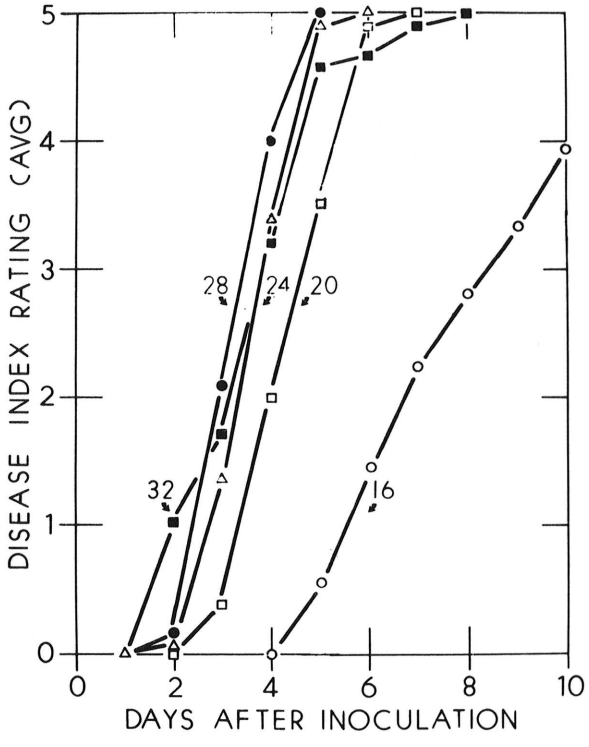

Fig. 2. Effect of temperature during incubation on development of anthracnose (caused by *Colletotrichum glocosporioides* f. sp. *aeschynomene*) in northern jointvetch. Plants were inoculated with 10^6 spores/ml, incubated for 24 hr in a dew chamber at 28 C, and then placed in a growth chamber and kept at the indicated constant temperatures. Disease index ratings are averages of three replications where 0 = no infection and 5 = plant death.

weed-control agent; i.e. (i) it sporulates abundantly in liquid culture, (ii) it is specific for species of *Aeschynomene,* and (iii) it rapidly kills or reduces weed stands in rice fields. We have found that temperature and moisture conditions prevailing in rice fields support rapid development of disease and subsequent control of the weed. Mean daily maximum temperatures in rice canopies are near 32 C from mid-June to mid-July in Arkansas, and the mean daily minimum air temperatures are near 24 C (2). Those temperatures, although not optimum for the disease, are sufficient for the disease to control the weed. Presumably, free moisture in rice fields also is sufficient for disease development; rice is continuously flooded during the time when weed control is needed and the RH is generally high enough to allow development of the pathogen. Applications of the pathogen in rice field environments have given excellent control of northern jointvetch (1). Results of our research in controlled-environment chambers indicate, however, that at least 8-12 hr of dew or free moisture were required for the fungus to infect all inoculated plants. An 8- to 12-hr dew period might be especially important if the inoculum contained fewer than 10^6 spores/ml.

Many pathogenic fungi require a period of free moisture for spore germination and infection. Wastie (13) concluded that moisture is the most important requirement for germination and infection by conidia of *C. gloeosporioides*. Others (6, 7, 9) have shown that several species of *Colletotrichum* penetrate their hosts within 9 to 12 hr after inoculation. Immediate penetration, however, may not be necessary for high levels of infection; Skoropad (10) reported that appressoria can remain dormant in unfavorable environments, and the stem epidermis is penetrated when the environment again becomes favorable. Northern jointvetch was 100% infected by *C. gloeosporioides* f. sp. *aeschynomene* with a dew period of only 12 hr which may be the amount of time required for the spores to germinate, produce appressoria, and penetrate the host tissue if temperature is optimal. Dormancy of the appressoria may explain the variations in the rate of lesion development or the differences in the number of lesions that develop under the various temperature regimes, because fewer penetrations occur when temperatures are less than the optimum.

Like other anthracnose diseases, northern jointvetch anthracnose develops rapidly over a wide range of temperatures (4, 5, 8, 12). However, development of the disease was slower in alternating temperature regimes, than at constant temperatures. An alternating day-night temperature of 32/24 C – only ± 4 C from the optimum temperature of 28 C – resulted in maximum inhibition of disease development. Similarly high or alternating temperatures slowed the development of lesions and subsequent disease symptoms of the rice blast disease caused by *Pyricularia oryzae* (3). Slowing of lesion development by the fluctuating temperatures which are common in field environments may reduce the efficacy of fungi for biological control of weeds. Significant variation in diurnal temperatures may reduce infection of weeds by the fungus or may increase the time required to kill.

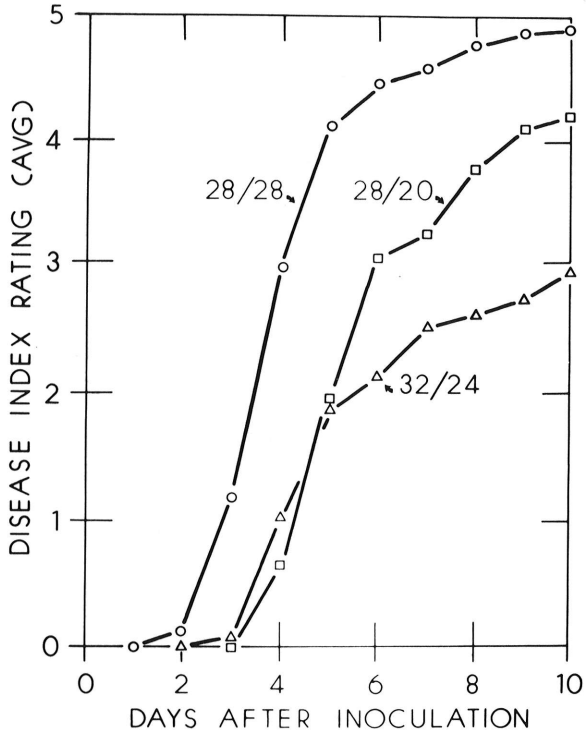

Fig. 3. Effect of alternating (day/night) temperature on development of anthracnose (caused by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*) in northern jointvetch. Plants were inoculated with 10^6 spores/ml, incubated for 12 hr in a dew chamber at 28 C, and then moved into a growth chamber at the day/night temperature regimes indicated. Disease index ratings are averages of three replications where 0 = no infection and 5 = plant death.

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