Effect of Temperature, Dew Period, and Inoculum Density on Blight of Safflower Caused by *Alternaria carthami*

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ABSTRACT

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The optimum temperature for expression of Alternaria blight symptoms on safflower (cultivar Gila) was 25 C. Frequency of stomatal penetration of leaf surfaces by germ tubes of *A. carthami* was greatest at 30 C. The percentage of safflower leaf area expressing blight symptoms increased as the length of the dew period and the inoculum density increased. Disease assessment keys based on the percentage of leaf or bract tissue showing symptoms of blight were developed to make quantitative disease assessments. A technique used to screen safflower cultivars for resistance to blight caused by *A. carthami* was also developed using the above results. Safflower seedlings were inoculated in a plastic tent with an inoculum density of 25×10^4 propagules of *A. carthami* per milliliter and incubated at 25 C for 24 hr.

Additional key words: *Carthamus tinctorius*

Leaf blight of safflower (*Carthamus tinctorius* L.) is caused by the fungus *Alternaria carthami* Chowdhury. Leaf blight is more severe in areas where high temperatures and high relative humidities prevail. The fungus can be seedborne or airborne and produces large, irregular lesions on the foliage, stem, and flower bracts of safflower (4). Crop losses resulting from the disease can be great. During the 1978–1979 growing season in Queensland, the disease was responsible for an estimated overall crop loss of 20%, with some crops experiencing 100% loss (5).

A satisfactory method of controlling safflower leaf blight has not been developed. Screening of cultivars for resistance to *A. carthami* under field conditions is unreliable because the level of natural infection depends on the prevailing environmental conditions, which may not be suitable for infection and disease development. Consequently, a reliable screening procedure is desirable to facilitate repeatable evaluation of disease resistance in safflower cultivars and lines to *A. carthami* in the glasshouse. The purpose of this investigation was to determine the effects of temperature, inoculum concentration, and duration of the dew period on disease to develop a method for screening safflower cultivars for resistance to *A. carthami* in a glasshouse. Disease assessment keys were developed to make quantitative disease assessments.

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MATERIALS AND METHODS

The safflower cultivar Gila was used in all experiments reported in this paper. Gila is the most popular commercial cultivar in Australia and is very susceptible to *A. carthami*. Three plants were grown in a soil-sand-peat (1:1:1) mixture contained in each 7.5-cm-diameter plastic pot. Plants were kept in a greenhouse at temperatures of between 24 and 30 C.

Plants at the six-leaf stage (4–5 wk old) were inoculated with an isolate of *A. carthami* that had been obtained from an infected safflower plant in the field. The inoculum consisted of a suspension of conidia and mycelial fragments contained in sterile water. Production of conidia by *A. carthami* on potato-carrot agar was enhanced by extensively cutting the mycelium of a 10-day-old culture and exposing the wounded mycelium to an alternating cycle of 12 hr light (6.5 $W \cdot m^{-2}$) and 12 hr darkness for 2 days. The inoculum was prepared by blending the wounded mycelium, conidia, and agar in a Sorvall Omnimixer (Ivan Sorvall Inc., Newton, CT). Mycelial fragments and conidia were used as the source of inoculum. We have observed that conidia and mycelial fragments germinate and penetrate the host tissue via stomates. The inoculum suspension was sprayed onto leaves using an Arnold precision touchup spray gun (Commonwealth Industrial Gases Ltd., Australia) at 300 kPa until incipient runoff.

Leaf samples were prepared for histological examination using the alcoholic-lactophenol cotton blue-chloral hydrate method (9). The percentage germination of propagules and the percentage of germ tubes that penetrated their host were determined 72 hr after inoculation. Twenty-five infective propagules (conidia and mycelial fragments) were examined on a single leaf segment sampled from each of three plants in each of five replicate pots.

The level of disease that developed in each treatment was determined by either counting the dead leaves or by using the disease assessment keys shown in Figures 1 and 2. The leaf blight assessment key was constructed by tracing the outline of a safflower leaf onto millimeter graph paper. The number of squares contained within the leaf outline was counted and diagrams with 0.5, 1, 5, 10, 25, 50, and 75% of the leaf area infected were calculated and drawn. A similar key was constructed to estimate the level of disease caused by *A. carthami* on safflower flower bracts. Both keys were based on the percentage of the host area that showed symptoms of disease, because this is usually considered the best means of assessing disease severity (6,7). In this study, both necrotic and chlorotic areas were assessed. Disease symptoms developed more slowly on the flower bracts than on leaves. Thus, disease ratings of the bracts were made 10 days after inoculation, whereas those on leaves were made 7 days after inoculation.

Effect of temperature on disease expression. Plants were inoculated with a suspension of 25×10^4 conidia and mycelial fragments per milliliter until incipient runoff. Development of the fungus and the expression of disease symptoms were studied at 10, 15, 20, 25, 30, and 35 C.

Two dew chambers (1) were used for this experiment. One, kept at 25 C, acted as a control with which infection at the other temperatures could be compared. The temperature of the second dew chamber was varied to each of the test temperatures. Plants were inoculated and placed in each dew chamber; they were incubated for 20 hr in darkness, then placed in a growth cabinet kept at 25 C and 43.5 $W \cdot m^{-2}$ light intensity (12-hr photoperiod). Disease ratings were made at each temperature 7 days after inoculation, using the disease assessment key shown in Figure 1 and by counting the completely necrotic and living leaves on each plant. Histological studies were

made on one randomly selected leaf sampled from each plant.

In a second experiment, the effect of postinfection temperature on disease expression was studied. Plants were inoculated as described before and incubated in a darkened dew chamber at 25 C for 20 hr. Five pots were then placed into each of six lighted growth cabinets (13 $W \cdot m^{-2}$ light intensity, 12-hr photoperiod) maintained at 10, 15, 20, 25, 30, and 35 C. Inoculated leaves were evaluated at 7 days for the percentage of leaf area showing disease symptoms. Completely necrotic leaves were also counted. One leaf segment from each plant was sampled for histological examination. Regression analysis was performed on the results of the postinfection temperature-disease relationship using the Bar 3 program (2,3) on the DEC-20 computer at the University of New England.

Effect of duration of dew period on disease expression. Plants were incubated in a plastic dew tent for 2, 4, 6, 8, 12, 16, 24, and 48 hr after inoculation with a spore and mycelial suspension (25×10^4 propagules per milliliter) until incipient runoff. The dew tent contained a Defensor humidifier (Defensor A.G. 8045, Zurich) and was located in a glasshouse where temperatures fluctuated between 24 and 30 C. At the end of each incubation period, five pots were removed from the tent and placed on a bench in the glasshouse. Disease expression was rated 7 days after inoculation. Regression analysis was performed on the data.

Effect of inoculum density on disease expression. Plants were inoculated with suspensions containing different densities of inoculum (1×10^4, 2.25×10^4, 6.25×10^4, 10.5×10^4, and 23.5×10^4 propagules per milliliter). Three plants in each of five pots were inoculated with each inoculum density to the point of incipient runoff. The pots were placed in a plastic dew tent for 18 hr and then on a glasshouse bench. The degree of disease development was rated 7 days after inoculation. Regression analysis was performed on the data.

RESULTS

Effect of temperature on disease expression. The percentage of leaf area showing disease symptoms and the proportion of dead leaves counted was greatest on plants inoculated at 25 C. The frequency of stomatal penetration was greatest at 30 C (Fig. 3). The second-degree polynomial was fitted to responses of the postinfection temperatures. Constant, linear, and quadratic coefficients were all significant at the 1% level. On differentiating and equating to zero, the optimum temperature was estimated to be 25.21 C (Fig. 4).

Effect of dew period on disease expression. Leaf blight symptoms were not observed on leaves subjected to dew periods of 2, 4, or 6 hr. After 6 hr of dew, a significant quadratic relationship ($r^2 = 0.95$) was observed between the natural logarithm of the duration of the dew period and the percentage of the leaf area showing symptoms (Fig. 5).

Effect of inoculum density on disease expression. There was a significant linear relationship ($r^2 = 0.90$) between the percentage of the leaf area showing symptoms and the natural logarithm of the inoculum density $\times 10^4$ (Fig. 6).

DISCUSSION

The environmental conditions for artificial inoculation that provided maximum infection by *A. carthami* on safflowers and the optimum expression of disease symptoms were a temperature near 25 C, a moisture period of at least 24 hr, and an inoculum concentration of about 23×10^4 propagules per milliliter. Our optimum temperature is similar to that reported by Savulescu (8), who demonstrated that 23 C was the optimum temperature for germination and infection by *A. carthami*. However, she found that germination was also high at 29 C.

The following method was developed to screen safflower plants for resistance to *A. carthami*: Plants were grown in a

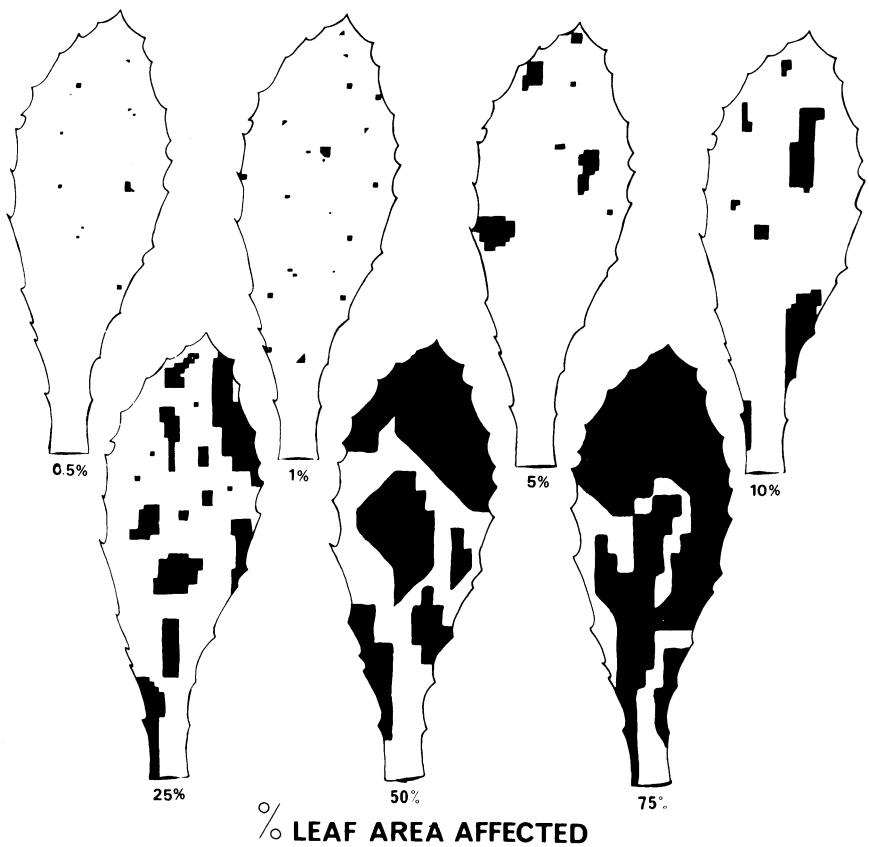

Fig. 1. Disease assessment key for blight (*Alternaria carthami*) on cultivated safflower.

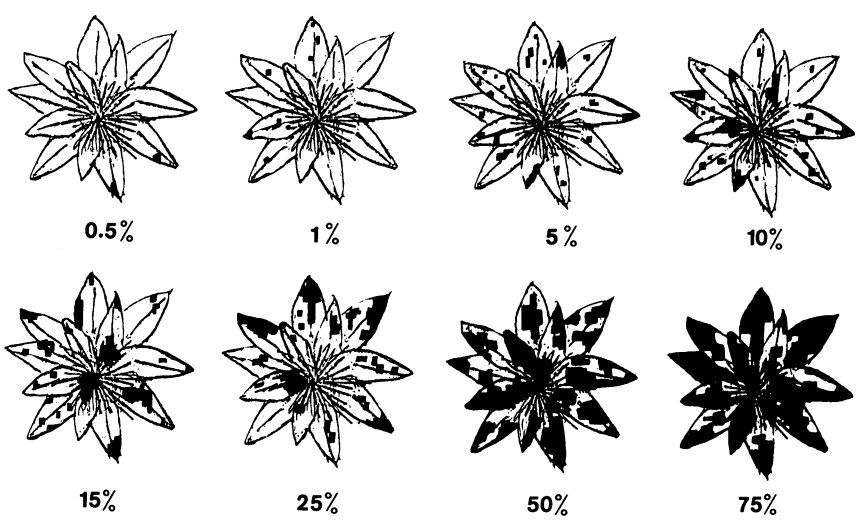

Fig. 2. Disease assessment key for leaf blight (*Alternaria carthami*) on flower heads of safflower.

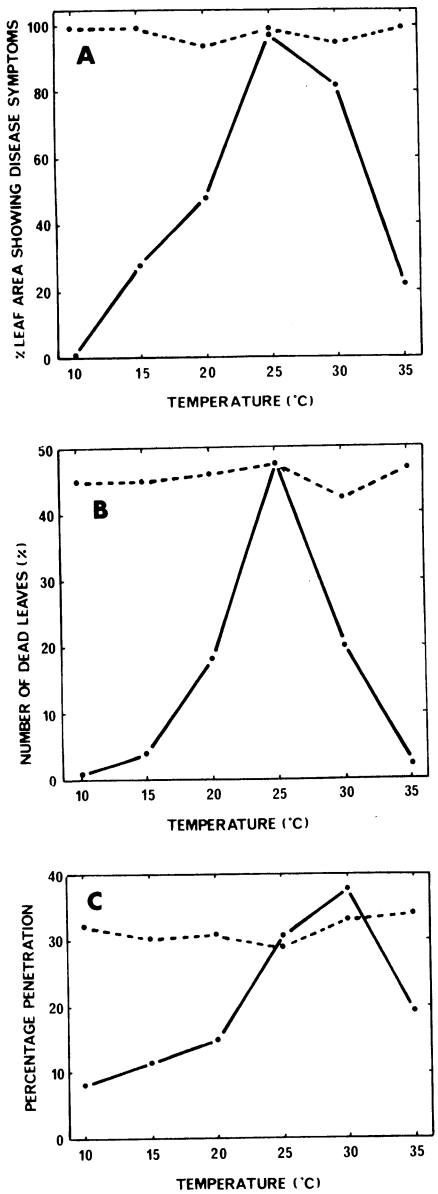

Fig. 3. Effect of a 20-hr postinoculation temperature on **(A and B)** disease expression and **(C)** frequency of penetration by germ tubes on safflower leaves inoculated with *Alternaria carthami*. ● - - - ● = Control plants kept at 25 C.

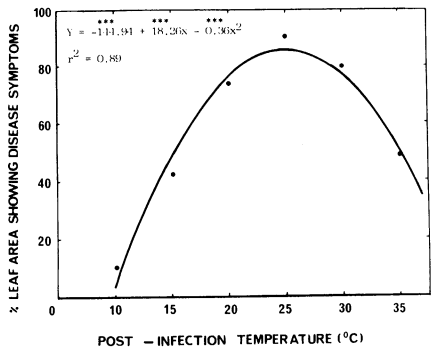

Fig. 4. Effect of postinfection (20 hr to 7 days) temperature on disease expression in safflower infected with *Alternaria carthami* (*** = $P > 0.001$).

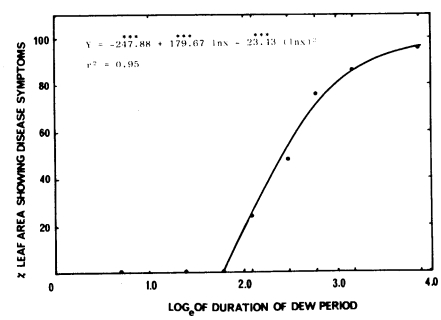

Fig. 5. Effect of duration of dew period during incubation on the amount of disease produced by *Alternaria carthami* on safflower (*** = $P > 0.001$).

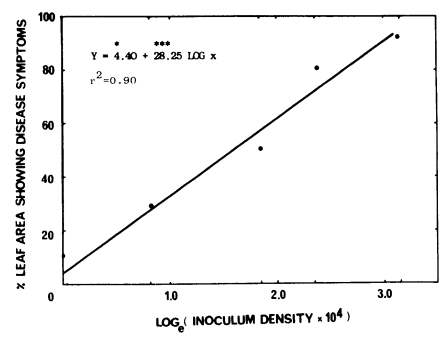

Fig. 6. Effect of inoculum density on leaf blight (*Alternaria carthami*) of safflower (cultivar Gila) (*** = $P > 0.001$).

glasshouse where temperatures were maintained at between 24 and 30 C. Benches were covered with a sheet of plastic to construct a tent over the bench. Overhead misters were installed to release a fine mist of water in the glasshouse to maintain high relative humidity during the incubation period. They ceased to operate once the plastic tent was removed, however, to ensure that the inoculum was not diluted or washed off the leaves.

Safflower breeding lines were placed in the "screening tent" with Gila plants included as susceptible controls. Pots and the inside surface of the tent were watered to increase the humidity. Plants were inoculated with a suspension containing 25×10^4 propagules per milliliter until the point of incipient runoff and incubated in the tent for 24 hr. The misters were then turned off and the plastic tent removed. Disease on leaves was assessed 7–10 days after inoculation, using the keys shown in Figures 1 and 2.

It was necessary to construct disease assessment keys for both the leaves and the bracts because four different reactions to infection by *A. carthami* were observed. Safflower plants may show resistant leaves and susceptible bracts, susceptible leaves and resistant bracts, susceptible leaves and bracts, and resistant leaves and bracts.

All breeding lines were inoculated and screened twice during the growing period, once at the six-leaf stage of growth and again at an advanced budding stage when bracts were prominent. More than 4,000 breeding lines have been screened for leaf blight resistance with this technique. About 7% of lines consistently show leaf blight resistance. This glasshouse disease resistance has been confirmed in the field in district trials conducted by the departments of agriculture in New South Wales and Queensland.

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