Biotic and Environmental Factors Affecting Infection of Sweet Corn with *Exserohilum turcicum*

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ABSTRACT

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Optimal temperatures for conidial germination and appressorial formation of *Exserohilum turcicum* on leaves of the susceptible corn cultivar Jubilee were 25 and 15 C, respectively, whereas that for infection peg formation on corn leaf impressions was 20 C. The minimal dew period required for these processes at the corresponding optimal temperatures was 1, 3, and 4 hr, respectively. Conidial germination and infection peg formation were not affected by leaf and plant age, but under laboratory conditions, appressorial formation was favored in younger plants that were also more susceptible to the blight incited by the fungus. Infection occurred at 15–30 C with an optimum at 20 C. A 5-hr dew period at 20 C was required for lesion formation. The number of lesions increased with lengthening dew period and increasing inoculum concentration, but under all combinations of dew period and inoculum concentration, the number of lesions was largest at 20 C. Our results indicate that environmental conditions in Israel during the growing season (May–October) are favorable for infection. Epidemic outbreaks of the blight do not occur until late August when dew periods are long enough for fungal sporulation.

Additional key words: epidemiology, northern corn leaf blight.

Northern leaf blight of corn incited by the fungus *Exserohilum turcicum* (Pass.) Leonard and Suggs is a major disease of sweet corn (*Zea mays* L.) (1). In Israel it reduces yields substantially late in the growing season (7). Although much is known about the effect of environmental and biotic factors on sporulation and spore dispersal of *E. turcicum* (6–9), little information is available about their effects on infection. Knox-Davies (5) showed that the pathogen penetrates corn leaves directly via an infection hypha developed from the lower surface of the appressorium. Once it is through the cuticle and outer epidermal cell wall, the fungus forms an intracellular vesicle. Occasionally subcuticular infection hyphae produce a few infection pegs.

In this article, we present data on the separate and combined effects of some environmental and biotic factors on the various phases in the infection process of corn by *E. turcicum* that shed some light on the epidemiology of the disease incited by the fungus.

MATERIALS AND METHODS

Plants and pathogen. Sweet corn (*Z. mays* 'Jubilee') was used in all experiments. Plants were grown as described earlier (8). Our culture of *E. turcicum* was isolated in 1978 from infected corn leaves collected in the coastal plain of Israel and propagated by repeated inoculations of corn plants in growth chambers.

Spore germination, appressorial formation, and infection peg formation. Spores were collected from sporulating corn leaves into water as described earlier (8) and calibrated to 10,000 spores per milliliter (unless otherwise stated) using a hemacytometer. For study of germination and appressorial formation, detached fourth corn leaves (from six-leaf plants) were laid on wet filter paper (adaxial surface upward) in plastic trays, sprayed with spore suspension, and covered with moistened polyethylene bags. Trays were then kept in the dark for the desired combination of time and temperature. At the end of the moist period, leaves were allowed to dry naturally (about 30 min) and a collodion imprint (about 4 cm^2) was taken from the middle part of each inoculated leaf (10 leaves per treatment). Imprints were stained with trypan blue (0.1%) in lactophenol and examined microscopically; 100 conidia were examined in each imprint. Conidia that had produced a germ tube of 25 μ were considered germinating.

Infection peg formation was investigated in vitro on collodion impressions. These were taken from the adaxial surface of healthy corn leaves and placed on wet filter paper in petri dishes. Impressions were sprayed with a conidial suspension of *E. turcicum*, kept at specific temperatures for desired periods, then stained with 0.1% trypan blue in lactophenol, and examined

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microscopically. Appressoria that had formed an infection peg were counted. Experiments were done in 10 replicates with 100 conidia counted per replicate.

Host penetration and infection. Infection peg formation in vivo seemed always to be associated with the collapse of epidermal cells beneath and near the appressorium, and by 48 hr after inoculation, such collapsed cells are visible as necrotic spots (10). Therefore, 48 hr after inoculation, we counted necrotic spots per unit leaf area to estimate the frequency of fungal penetration into leaf tissue. Disease development was determined 7 days after inoculation by determining the percentage of necrotic leaf area (using tracing on transparent paper and weighing).

Effect of dew period, dew temperatures, and inoculum concentration. The infection of four-leaf corn plants by *E. turcicum* was investigated under controlled conditions in a $6 \times 3 \times 4$ factorial experiment with 72 combinations of dew period, dew temperature, and inoculum concentration. Plants were inoculated by spraying them with inoculum suspensions of 1,500, 5,000, or 10,000 conidia per milliliter, covered with moist plastic bags, and incubated in the dark at leaf temperatures of 10, 15, 20, 25, 30, or 35 C (all ± 1 C) for 3, 6, 9, or 12 hr. Leaf temperature was measured within the moisture chambers by attaching small thermocouples to leaves. At the end of the dew period, plants were exposed and allowed to dry naturally (about 30 min), then transferred to a 25 C cabinet (50–60% RH) illuminated with cool-white fluorescent lamps supplemented with incandescent bulbs for 12 hr/day at an intensity of about 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Lesions per plant were counted 1 wk later.

RESULTS

Conidial germination on corn leaf surfaces. Spores germinated in the presence of free moisture in the dark at temperatures of 10–35 C with an optimum at 25 C (Fig. 1). Germ tubes grew only from terminal cells of conidia. Percentage germination was not affected by plant age (9–47 days) or by leaf age (leaf 1–8; data not presented). No germination occurred on leaves irradiated with 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ at 20–25 C, confirming our previous observations (10).

Formation of appressoria on corn leaves. On 2% water agar, conidia germinated abundantly but produced no appressoria. Appressoria formed abundantly on leaf surfaces and on collodion imprints of corn leaves. Percentages of appressorial formation on 9-, 29-, and 47-day-old plants were 80–90, 65–82, and 30–50%, respectively. Small (mostly nonsignificant) differences in the frequency of appressorium formation were observed among leaves of different ages on the same plant (Fig. 2). The percentage of leaf area blighted in these plants (Fig. 2, insert) was proportional to percentage conidia producing appressoria.

Two temperature optima were observed for appressoria formation. After 3 hr of dew, leaves incubated at 25 C had the most

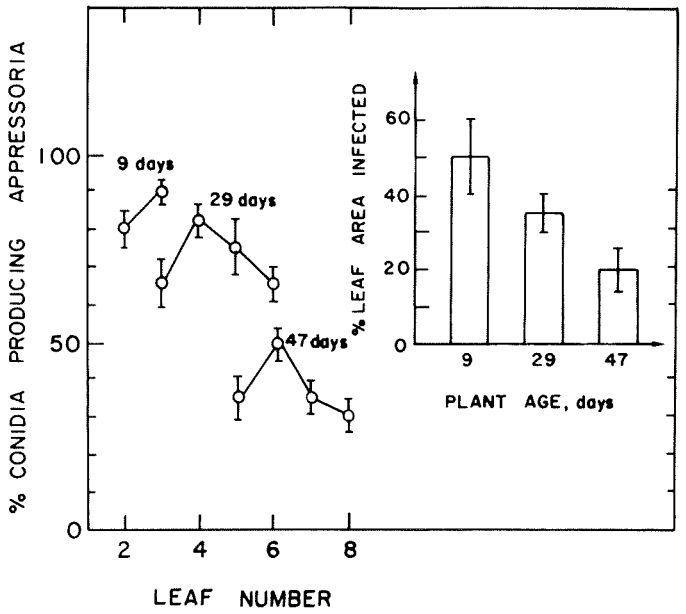

Fig. 2. Effect of leaf and plant age on appressorial formation of *Exserohilum turcicum* at 20 C and 9 hr of dew in the dark. Insert: Percentage leaf area blighted in the same plants 7 days after inoculation. Bars represent standard deviations of the mean (n = 10).

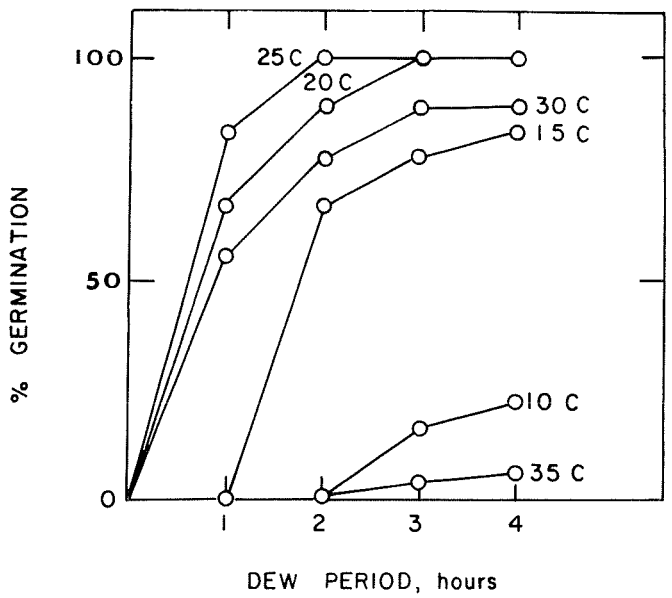

Fig. 1. Effect of temperature and dew period on conidial germination of *Exserohilum turcicum* on corn leaves in the dark. Average standard deviation for all treatments was 9%, with a range of 5–14% (n = 10).

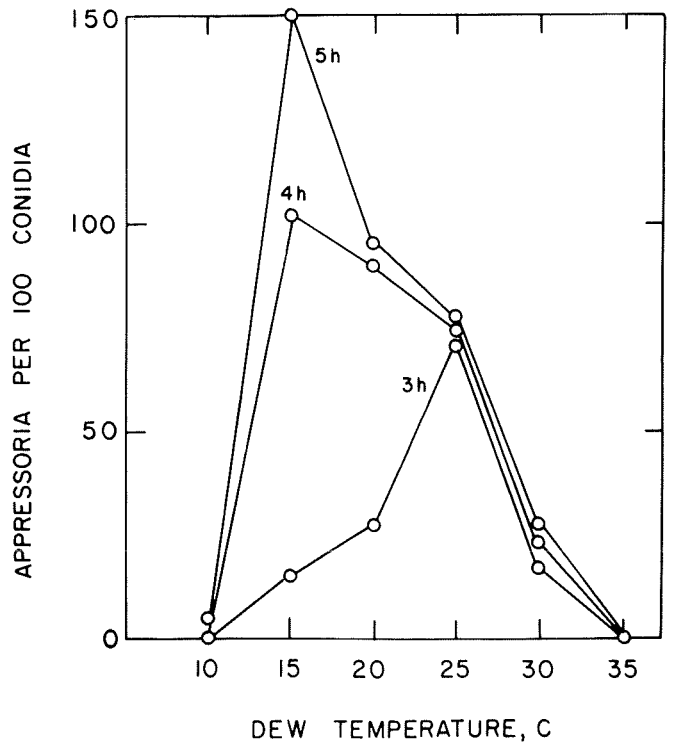

Fig. 3. Effect of temperature and dew period on number of appressoria of *Exserohilum turcicum* formed on corn leaves in the dark. Average standard deviation for all treatments was 17%, with a range of 5–25% (n = 10).

appressoria, whereas after 4 or 5 hr of dew, leaves incubated at 15 C had the most (Fig. 3). Although germination (Fig. 1) and appressorial formation were slower at 15 than at 25 C, more than twice as many appressoria were ultimately produced at 15 than at 25 C (Fig. 3), owing to the fact that 58% of conidia at 15 C produced two appressoria compared to 8% at 25 C.

Infection peg formation on corn leaf imprints. Infection peg formation on collodion impressions taken from corn leaves was first detected after 4 hr of dew, remained relatively slow at all temperatures up to 6 hr after inoculation, and then proceeded at the fastest rate at 20 C (Fig. 4).

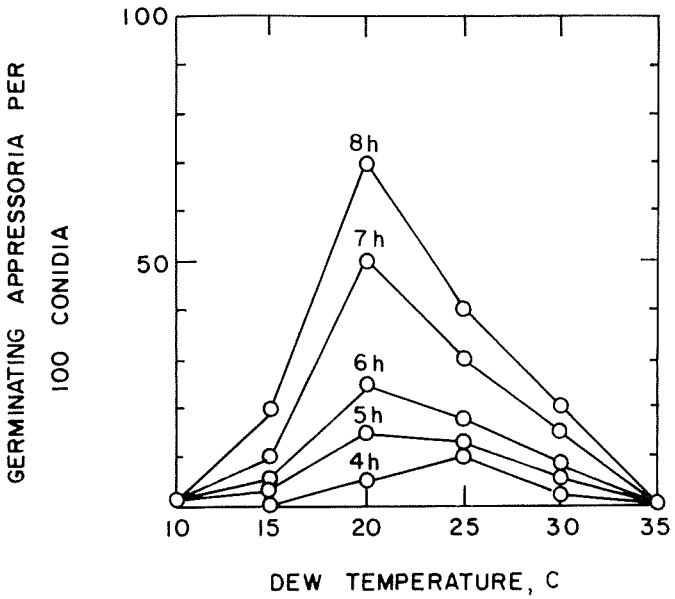

Fig. 4. Effect of dew temperature and dew period on appressorial germination of *Exserohilum turcicum* on collodion corn leaf impressions. Average standard deviation for all treatments was 12%, with a range of 6–17% (n = 10).

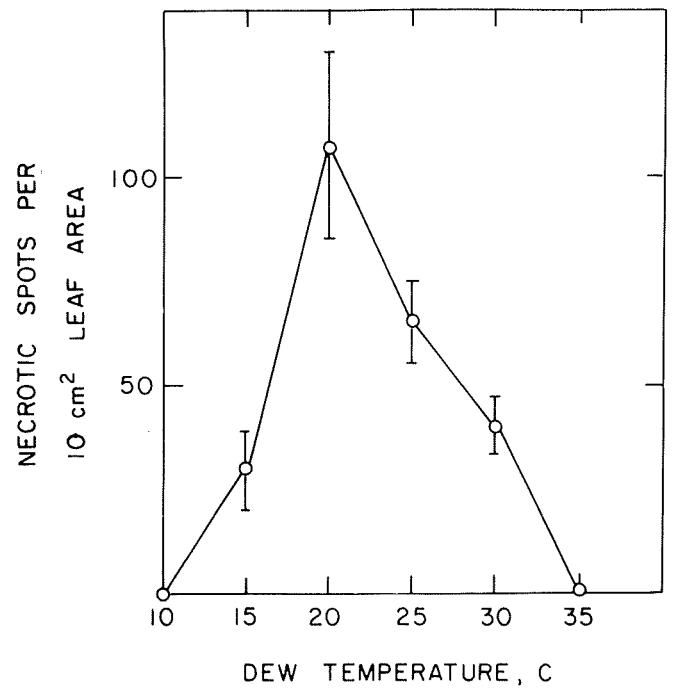

Fig. 5. Number of necrotic spots formed on corn leaves incubated for 9 hr after inoculation with *Exserohilum turcicum* at various dew temperatures in the dark and then transferred for 48 hr to 25 C with a photoperiod of 12 hr/day. Bars represent standard deviation of the mean (n = 10).

Fungal penetration into corn leaves. As evidenced by necrotic spot formation, after 9 hr of dew, penetration occurred from 15 to 30 C with an optimum at 20 C (Fig. 5). In spite of the high frequency of appressorium formation at 5 hr at 15 C (Fig. 3), only 30 necrotic spots per 10 cm^2 of leaf area were counted at this temperature (Fig. 5) due to a low proportion of appressorial germination at this temperature (Fig. 4). When inoculated plants were kept wet for 6 hr at 15 C and then for 3 hr at 25 C, the number of necrotic spots produced increased markedly (95/10 cm^2) compared to 9 hr at 15 C (30/10 cm^2).

Effect of dew period, dew temperature, and inoculum concentration. For all combinations of dew period and dew temperature, the number of lesions per plant 1 wk after inoculation increased with increasing inoculum concentration (Fig. 6). No lesions formed after a 3-hr dew period at any combination of inoculum concentration and dew temperature. As dew period increased from 6 to 12 hr, the number of lesions per plant increased, reaching a maximum of 70 lesions per plant at 20 C, 12 hr of dew, and 10,000 conidia per milliliter. Regardless of dew period duration and inoculum concentration, maximal infection occurred at 20 C. The range of temperatures at which infection took place varied according to the inoculum concentration and the length of the dew period. At marginal conditions of dew period and inoculum concentration (6 hr, 1,500 spores per milliliter), infection occurred at 20–25 C, with traces at 30 C, whereas with longer dew periods and higher inoculum concentration, infection occurred at 15–30 C. No infection took place at 35 C.

DISCUSSION

Here and previously (10), we have demonstrated that infection of corn with *E. turcicum* is influenced by three environmental and two biotic factors: light, dew temperature, dew period, plant age, and inoculum concentration. This information may be valuable for simulating blight development and for making decisions about disease control.

Providing that inoculum is present, the most important factor influencing infection is dew period duration. Five hours of dew at optimal temperature was the minimum for establishing infection (2). Infection peg formation and lesion development were most frequent at 20 C, whereas appressorial formation was most frequent at 15 C. Differences in optimal temperature requirements for different stages of pathogenesis are similar for *Helminthosporium maydis* (11,14).

The dew temperature range in which infection occurred depended largely on the inoculum concentration and length of the dew period (Fig. 6). When the latter two factors were marginal, infection occurred only in a narrow temperature range of 20–25 C, whereas when they were favorable, infection occurred over the wider range of 15–30 C. Similar findings on the relative limits of inoculum loads, wetting periods, and temperatures on infection were reported before (3,13).

Young plants are more susceptible than older plants to infection by *E. turcicum* owing to their stimulating effect on appressorial formation (Fig. 2). Similar results were reported by Ying (15) for infection of corn by *H. maydis* and by Hau and Rush (4), who showed that cultivar resistance in rice against *H. oryzae* was largely attributed to a low frequency of appressorial formation. In Israel, epidemic outbreaks of northern leaf blight occur in relatively older plants (about 50 days), but we have observed that when inoculum is abundant, younger plants suffer much greater damage than do older plants.

As for many other plant diseases (12), the minimal dew period required for sporulation of *E. turcicum* on corn is much longer (14 hr) (8) than that required for infection (5 hr). Because in Israel dew is less frequent in spring and early summer than in late summer, we concluded that *E. turcicum* can hardly complete its life cycle in the former seasons (dew periods are too short to induce sporulation) and therefore epidemic outbreaks are rare. In late summer and autumn, the dew periods lengthen, sporulation is abundant, inoculum potential increases, and epidemic outbreaks are frequent.

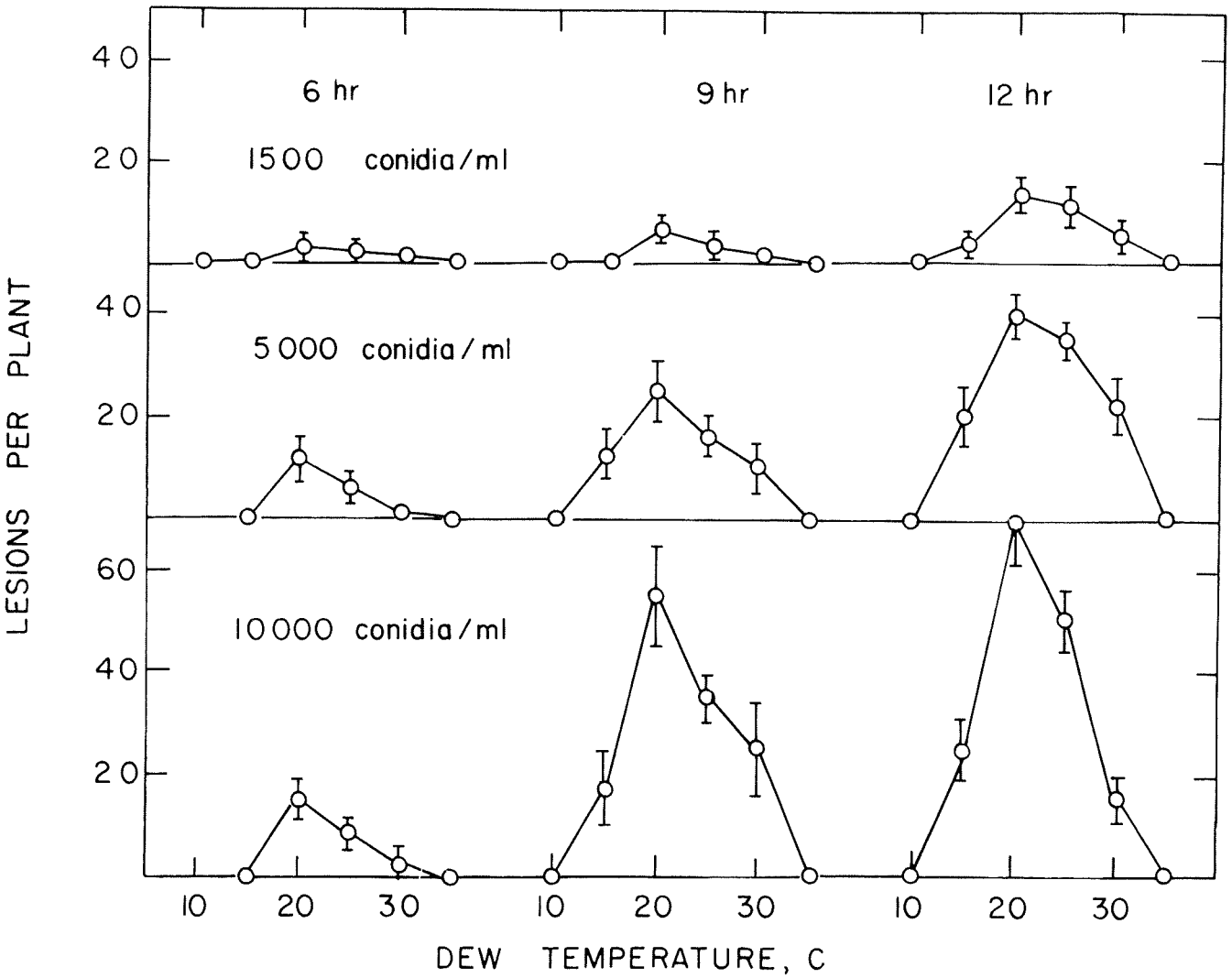

Fig. 6. Combined effect of dew temperature, dew period, and inoculum concentration on infection of sweet corn by *Exserohilum turcicum*. Bars represent standard deviation of the mean (n = 10).

This conclusion agrees with that of Berger (2), who found that a daily average of 6.5 blight favorable hours (RH is near 100% and temperature above 15 C) caused too little blight to justify fungicide application, but 11 blight favorable hours caused an epidemic regardless of fungicide application.

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