Differential Effect of Light on Spore Germination of *Exserohilum turcicum* on Corn Leaves and Corn Leaf Impressions

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ABSTRACT

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The effect of light on spore germination of *Exserohilum turcicum* was investigated in vitro on collodion corn leaf impressions and in vivo on leaf surfaces of susceptible corn plants. On collodion impressions, the percentage spore germination under light at a photon flux density of 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ was the same as in darkness. On host leaf surfaces, light at a photon flux density of 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$ inhibited spore germination by 85% (at 20 C) compared to the dark control. Blue light was more inhibitory than red light to spore germination in vivo. Inhibition under light persisted in the presence of dichlorophenyldimethylurea, a photosynthesis inhibitor. As a result of the inhibition of spore germination, no infection occurred on leaves irradiated during the wet infection period. It is suggested that light inhibited spore germination on the leaf surface indirectly by some physiological change in the host.

Additional key words: *Helminthosporium turcicum*, northern corn leaf blight.

The effect of light on fungal spore germination has been studied extensively by many workers (2,3). However, most have restricted their studies to germination in vitro (7,11). Only a few have extended their studies to the in vivo situation using plant material as a substrate for germination. Knights and Lucas (9) recently showed that light inhibited spore germination of *Puccinia graminis* f. sp. *tritici* either in vitro or in vivo.

In this paper we provide evidence that light has a different effect on spore germination in vivo compared to in vitro. Dissimilarity between in vivo and in vitro response has previously been shown by Rotem et al (12) for the morphological processes associated with fungal sporulation.

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MATERIALS AND METHODS

Plants, pathogen, and inoculation. Susceptible sweet corn (*Zea mays* L. 'Jubilee') was used in all experiments. Plants were grown as described earlier (10). *Exserohilum turcicum* (Pass.) Leonard and Suggs was isolated from infected corn leaves collected in 1979 from a commercial corn field in the coastal plain of Israel, and propagated on corn leaves by repeated inoculations in growth chambers.

For germination studies, spores were collected by brushing spores from sporulating leaves into water with the aid of a camel's hair brush and filtering through three layers of cheesecloth. Concentration was adjusted to 10,000 spores per milliliter based upon counting with a hematocytometer. Corn leaf impressions were prepared from the adaxial leaf surfaces (leaf No. 4 of seven-leaf plants) by using collodion solution (10% collodion powder 950, Merck, in ether:ethanol [3:1]). Detached corn leaves (adaxial surface upward) and impressions were placed on moist filter paper

in plastic trays, sprayed with a spore suspension, covered with transparent polyethylene bags (0.01 mm), and incubated in the dark or under lights for the desired period of time in growth cabinets at the appropriate temperatures. Temperature at the leaf or collodion surface was measured with a small thermocouple attached to it. White light was supplied from very high output, cool-white Sylvania F48T12 fluorescent lamps and blue or red light from General Electric model F40B fluorescent lamps with maximal emission spectra at 450 nm range 350–650 nm and at 640 nm from a General Electric model F40R, range 600–700 nm lamp, respectively (General Electric, Nela Park, Cleveland, OH 44112) (5). Light intensity was measured at leaf level by using a LI-185 quantum meter (Lambda Instruments, Lincoln, NE 68504).

At the end of the germination period, leaves and collodion leaf impressions were exposed and dried for 30 min; the impressions were stained with trypan blue (0.1%) in lactophenol and examined microscopically.

To observe germination on leaves, collodion impressions were taken at the end of the germination period, stained similarly, and examined microscopically.

In vivo appressorium formation was always associated with collapse of epidermal cells beneath and close to the appressoria. At 48 hr after inoculation, the collapsed cells were visible as necrotic spots. Number of necrotic spots per unit leaf area was used as a criterion for fungal penetration into leaf tissue. In some experiments, the healthy and the infected leaf areas were measured by tracing on transparent paper, and percentage of leaf area infected was calculated.

RESULTS

Light (cool-white fluorescent, 150 $\mu E \cdot m^{-2} \cdot sec^{-1}$) inhibited spore germination on corn leaves, but not on collodion impressions taken from corn leaves. In the dark, percentages of germination were 90 ($\pm$12) and 87 ($\pm$19) on leaf surface and collodion impressions, respectively, as against 15 ($\pm$9) and 85 ($\pm$17) on leaf surface and collodion impressions, respectively, incubated under light. The morphological characteristics of germinated conidia under light and darkness on corn leaves and on leaf impressions are shown in Fig. 1. Conidial germination on corn leaves irradiated with increasing levels of cool-white fluorescent light is given in Fig. 2.

Blue light was more inhibitory (68%) than red light (14%) to spore germination on corn leaves (Table 1). The inhibitory effect of light on conidial germination in vivo was dependent upon temperature. Under a low level of blue light (40 $\mu E \cdot m^{-2} \cdot sec^{-1}$) inhibition was detected at 20 and 25 C, but not at 15 and 30 C, whereas at a high light level (90 $\mu E \cdot m^{-2} \cdot sec^{-1}$) inhibition was noticed at all temperatures tested (Fig. 3).

Under all combinations of light and temperature, no greater inhibition was observed on collodion leaf impressions than on the

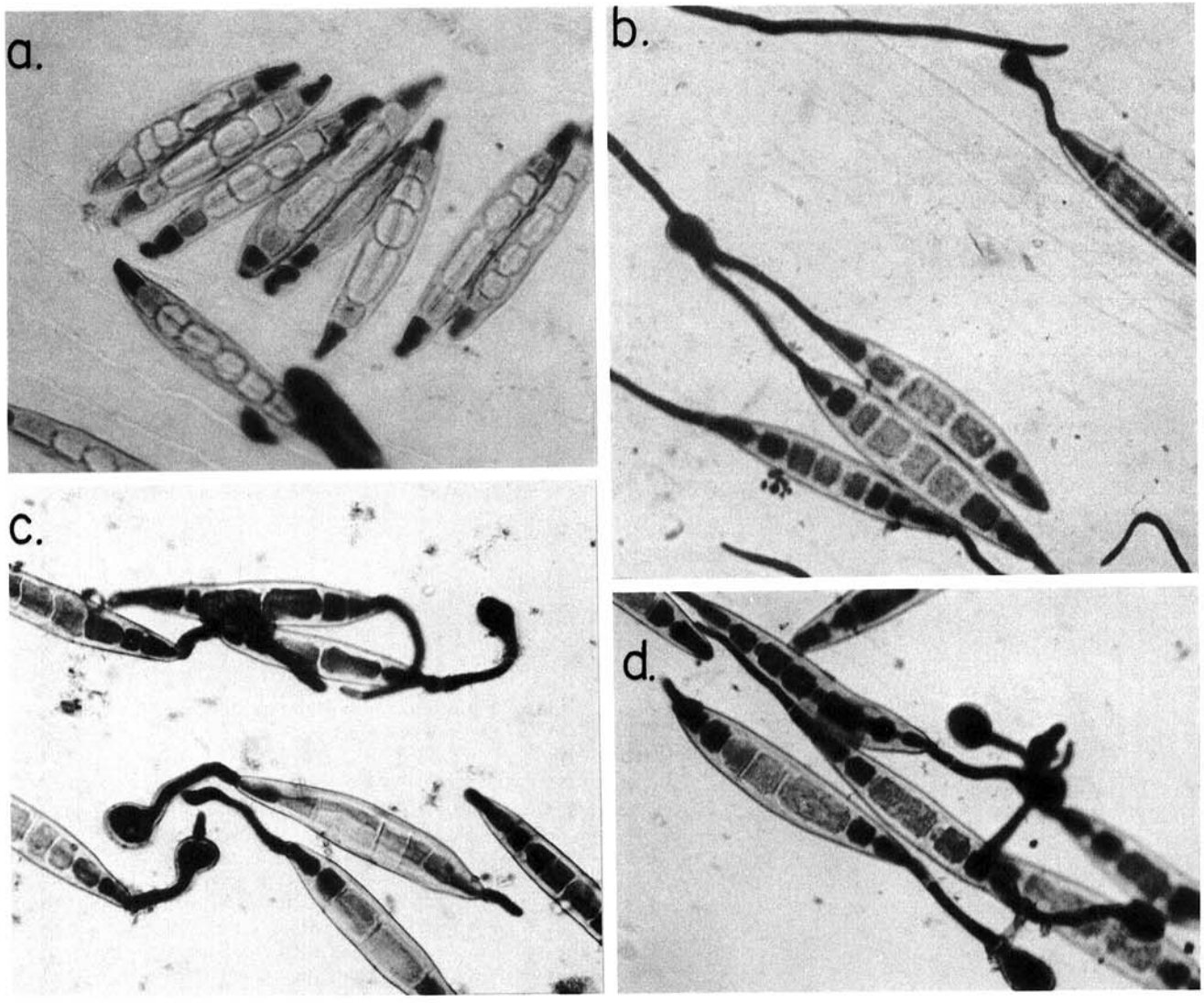

Fig. 1. The morphological characteristics of conidial germination of *Exserohilum turcicum* on corn leaves and on corn leaf impressions under cool-white fluorescent light (150 $\mu E \cdot m^{-2} \cdot sec^{-1}$) and darkness at 20 C (7 hr): **a,** Germination under light in vivo; **b,** Germination in darkness in vitro; **c,** Germination under light in vivo; and **d,** Germination in darkness in vitro.

corresponding dark controls.

The period of spore germination sensitive to irradiation was determined by transferring inoculated detached leaves from light to darkness and vice versa at various intervals after inoculation. Two hours of initial incubation under light followed by 5 hr of incubation in darkness inhibited germination by 82% (Table 2). Two hours of initial incubation in darkness followed by 5 hr of incubation in light inhibited germination by 29%, indicating that the first 2 hr of germination is the period sensitive to irradiation.

Dichlorophenyldimethylurea (DCMU) at 10^{-4} M applied to corn leaves before inoculation or during the germination period did not diminish the inhibitory effect of light on conidial germination (in the dark, 90 [±8] and 85 [±11] % germination on water-treated and DCMU-treated leaves, respectively, and under light 10 [±12] and 15 [±10]% germination on water-treated and DCMU-treated leaves, respectively).

Exposure of inoculated corn plants to light during the wet infection period reduced subsequent disease development compared to incubation in darkness (Fig. 4). Exposure to light before inoculation had no effect on infection if the wet infection period was conducted in darkness (*unpublished*).

DISCUSSION

Fungal plant pathogens may be classified into three main groups based on the effect of light on spore germination: light has no effect (eg, *Erysiphe cichoracearum* [Y. Cohen, *unpublished*]); light is necessary for germination (eg, *Physoderma maydis* [8]); light inhibits germination (eg, *Puccinia graminis* f. sp. *tritici* [9]).

This paper shows that light (mostly blue) had no direct effect on spore germination of *Exserohilum turcicum*, but inhibited it indirectly through physiological activity of the host. The inhibitory effect of light was temperature dependent and reached its maximum at 20–25 C, a temperature range that also was optimal for germination in darkness at 3 hr (*unpublished*).

This interaction between the effects of light and temperature on spore germination is difficult to explain. A possible explanation is that a relatively high temperature (20–25 C) is required for enzymic buildup of antifungal compound(s) by the host, but because of this compound's volatility or thermalability it is less inhibitory at 30 C. Volatile compounds are known to affect germination of urediospores of *Puccinia graminis* on wheat (6).

The rapid response of corn leaves to the presence of the pathogen

TABLE 1. The effect of blue and red light (both at 40 $\mu E \cdot m^{-2} \cdot sec^{-1}$) on germination of *Exserohilum turcicum* on corn leaves and on collodion leaf impressions (25 C, 7 hr)

Treatment	Substrate	Germination[a] (%)
Blue light	Leaf	30 a
	Leaf impression	85 b
Red light	Leaf	80 b
	Leaf impression	83 b
Dark control	Leaf	93 b
	Leaf impression	87 b

[a] Different letters indicate significant ($P < 0.01$) differences according to Duncan's multiple range test.

TABLE 2. Conidial germination of *Exserohilum turcicum* on corn leaves transferred from darkness to light (cool-white fluorescent, $\mu E \cdot m^{-2} \cdot sec^{-1}$), and vice versa at 1 or 2 hr after inoculation (25 C)

Treatment	Germination[a] (%)
Continuous light (7 hr)	17 a
2 hr light — 5 hr darkness	25 a
1 hr light — 6 hr darkness	45 b
2 hr darkness — 5 hr light	62 c
1 hr darkness — 6 hr light	40 b
Continuous darkness (7 hr)	87 d

[a] Different letters indicate significant ($P < 0.01$) differences according to Duncan's multiple range test.

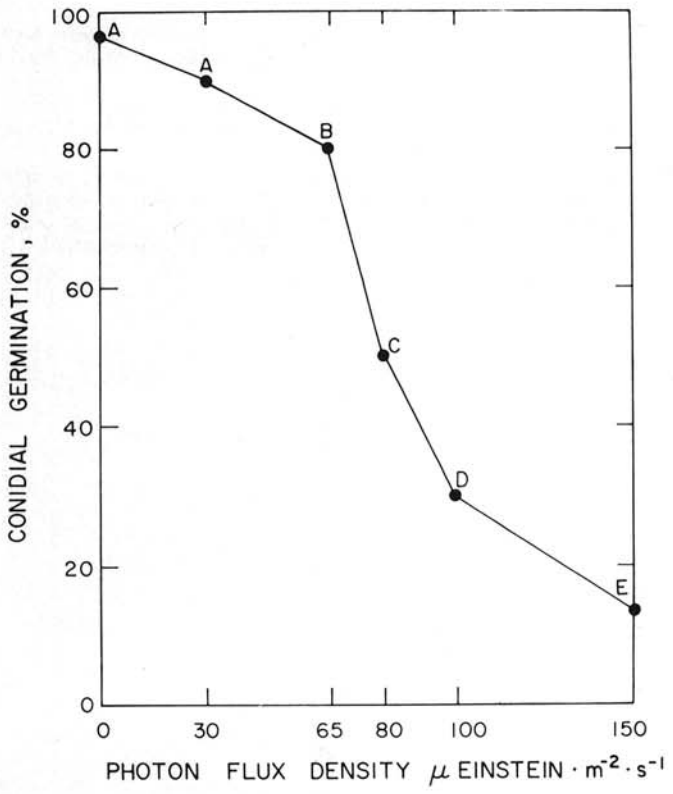

Fig. 2. Conidial germination of *Exserohilum turcicum* on corn leaves irradiated with increasing levels of cool-white fluorescent light at 20 C. Different letters on curve indicate significant ($P < 0.01$) differences according to Duncan's multiple range test.

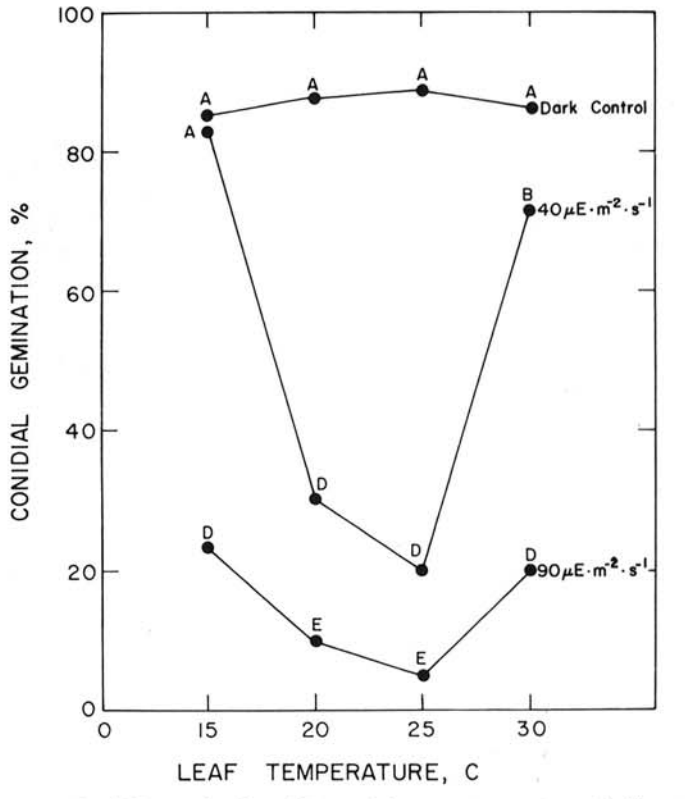

Fig. 3. Conidial germination of *Exserohilum turcicum* on corn (cultivar Jubilee) leaves under various combinations of temperature and blue light (7 hr). Different letters on curves indicate significant ($P < 0.01$) differences according to Duncan's multiple range test.

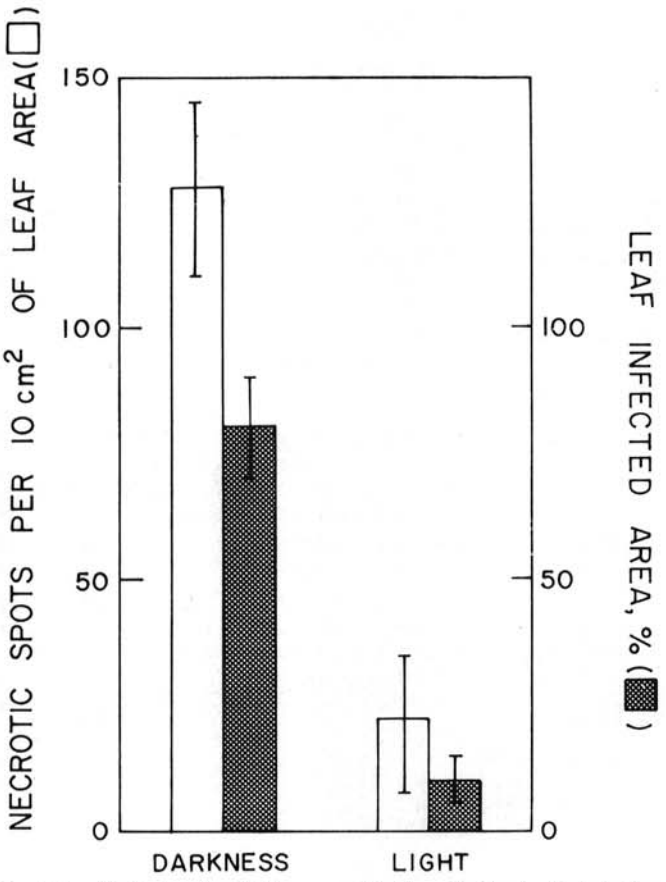

Fig. 4. The effect of cool-white fluorescent light applied during the infection period (8 hr, 20 C) on disease development in intact corn plants inoculated with *Exserohilum turcicum*. Shaded columns represent percentage infected leaf area measured at 7 days after inoculation. Open columns represent number of necrotic spots per unit leaf area counted at 48 hr after inoculation.

is hard to accept. Nevertheless, it stands in accordance with the finding of Cohen and Eyal (4) who showed that uredospores of *Puccinia sorghi* probably induce the secretion of antifungal compound(s) when allowed to germinate for 2 hr on corn leaves (cultivar Jubilee). It appears that the physiological activity of the host that influences germination is not connected with photosynthesis because DCMU, which blocks photosynthesis (13, and *unpublished* results, which showed that $^{14}CO_2$ incorporation into DCMU-treated corn leaves was inhibited 99%), did not prevent the inhibitory effect of light on germination and because red light had no inhibitory effect on germination. Possibly other blue light photoreceptors, which are known to exist in corn plants (1), are involved in the process of inhibition.

The inhibitory effect of light on germination in vivo may bear significant epidemiological consequences. Free leaf moisture persisting during the day would induce much less infection than moisture at night.

Such information on the effect of light on conidial germination and infection may be valuable for simulation models of the northern leaf blight of corn.

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