Resistance of Maize to Anthracnose : Effect of Light Intensity on Lesion Development

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ABSTRACT

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The size of anthracnose lesions on leaves of corn lines resistant or hypersensitive-resistant to *Colletotrichum graminicola* was significantly decreased under high light intensity (approximately 37,600 lux). However, with the exception of the inbred K4, lesion size on anthracnose susceptible corn lines was not significantly reduced. Lesion size was similar on susceptible and resistant lines grown under low light intensity (approximately 9,600 lux), but lesion type differed irrespective of light intensity. The significant decrease in lesion size on the resistant and hypersensitive-resistant lines, and on the susceptible inbred K4, was associated with the accumulation of anthocyanin pigments around the lesions.

Additional key words: phenolic metabolism.

Light has been reported to affect symptom expression in many host/pathogen interactions. For example, high light intensity and longer photoperiods decreased lesion size and/or numbers in infections due to *Phytophthora infestans* (13) and *Alternaria solani* (1) on potato leaves, *Phytophthora drechsleri* on safflower (11), and *Helminthosporium turcicum* on corn (5). However, the size of lesions caused by *Cladosporium fulvum* on tomato was increased under high light intensity (4). It has been reported that higher light intensities caused an apparent decrease in disease severity on corn infected by *Colletotrichum graminicola* (Ces.) Wils. (7, 14).

Thompson and Leonard (12) used lesion length for evaluation of resistance of corn to *C. graminicola*. However, Nicholson and Warren (6) proposed the use of lesion type rather than size for evaluation of resistance of corn to anthracnose. The lesion type for susceptible corn lines was defined as typically oval, with a gray-green color on both leaf surfaces, and with concentric zones in enlarged lesions. Resistant and hypersensitive-resistant lesion types were brown to tan and often were surrounded by chlorotic or yellow-orange discolored zones at the lesion margin. The hypersensitive-resistant lesion type rarely became larger than the original chlorotic flecks which were evident about 42 hr after inoculation (6).

Since lesion type, rather than size, was characteristic of anthracnose resistance and susceptibility (6), and light intensity was reported to affect disease severity (7, 14),the influence of light intensity on lesion type and size was investigated.

MATERIALS AND METHODS

Colletotrichum graminicola, isolate 104, isolated from diseased corn leaves from Indiana (6), was maintained on oatmeal agar in the light (approximately 3,228 lux) at 24 C. Spore suspensions for inoculations were prepared as previously described (6) using 2-wk-old cultures. The spore concentration was adjusted to 7.5×10^5 spores/ml, and one drop of Tween-80 was added per 100 ml of suspension. The lines of corn (*Zea mays* L.) used were the inbreds A632, CI74, K4, Mo17, Mo940, 33-16, Purdue dent hybrid PM [PM= (K61 × K64) × CI74], and the sweet corn hybrid Deep Gold (Asgrow Seed Co., Kalamazoo, Mich.). The lines Mo940, Deep Gold, Mo17, and K4 are susceptible to *C. graminicola,* A632 and CI74 are resistant, and PM and 33-16 are hypersensitive-resistant (6). Two replicate sets of four seedlings per corn line were grown under high (approximately 37,600 lux) or low (9,600 lux) light intensities in growth chambers at approximately 22.5 C with a 12-hr photoperiod. Seedlings were inoculated when the third leaf above the plumular leaf had fully emerged (16 days after planting). Plants were inoculated in the greenhouse by spraying the inoculum onto the leaves with an atomizer pressurized at approximately 0.5 atmosphere. Plants were incubated in a humidity chamber for 18 hr and then returned to growth chambers.

The size of seven lesions from the fourth leaf of each of four plants (28 lesions per corn line per replicate) was recorded by tracing lesion outlines at 8 days after inoculation. The tracings were photographed, enlarged, and lesion areas (mm^2) were determined with a polar planimeter. Comparative differences in lesion sizes

produced under high and low light intensities for each host and between hosts within each light treatment were statistically analyzed by the Newman-Keuls sequential range test.

Extraction of anthocyanins from tissue surrounding developing anthracnose lesions.—Lesion sites were removed from leaves of corn lines PM (hypersensitive-resistant) and Mo940 (susceptible) with a cork borer (5-mm internal diameter) 3.5 days after inoculation. At this time lesions on both corn lines were less than 2 mm^2. Therefore, the leaf disks sampled were composed primarily of tissues which were not part of the developing lesions. Leaf disks were taken from the fourth leaf of plants grown under both high and low light intensities. Noninoculated plants served as controls. Leaf disks were weighed and placed in 10 ml of boiling methanol per gram fresh weight (gfw) for 5 min. The methanol was decanted and the tissue was homogenized in acidified (0.1% HCl) 80% methanol (10 ml/gfw), centrifuged (2,000 *g*, 5 min), and the pellet was extracted twice with acidified methanol. The methanol extracts were combined and concentrated to near dryness by flash evaporation at 32 C. The residue was suspended in distilled water (5 ml/gfw) and extracted twice with hexane (v/v). The water fraction was then extracted with petroleum ether (1:1, v/v), placed under vacuum to remove residual petroleum ether, and extracted (v/v) with ethyl ether (8). The remaining water fraction was brought to a concentration ratio of 2 ml/gfw. Paper chromatography was carried out by applying 500 μliters of the water extract as a 5-cm band to Whatman 3 MM paper and developing in an ascending solvent of butanol: acetic acid: water (4:1:5, v/v) at 24 C (3). Absorption maxima were measured after diluting the water extract 50-fold with distilled water (2).

RESULTS

Lesions on corn lines resistant or hypersensitive-resistant to *C. graminicola* were significantly reduced in size under high light intensity. Lesion size on susceptible lines was not affected by light intensity, with the exception of inbred K4 (Table 1). Hypersensitive-resistant and resistant lines exhibited a decrease in average lesion size of 2.38 to 2.45 mm^2 and 3.49 to 5.61 mm^2, respectively, under high light intensity. The lesion type characteristic of each corn line (6) was not affected by light intensity. Thus, resistant, hypersensitive-resistant, and susceptible lines exhibited their respective lesion types under both the high and low light intensities.

A red pigment accumulated in tissues surrounding lesions on each of the resistant and hypersensitive-resistant lines and in the susceptible inbred K4 when grown under high light intensity (Fig. 1-a). Also, lesion size was significantly decreased in lines which accumulated the red pigment. No pigment accululated, and lesion size was not significantly reduced, in any corn line when grown under low light intensity (Fig. 1-b and Table 1). The water-soluble extract from the inoculated PM corn line (grown under high light intensity) was a bright red color, whereas similar extracts from inoculated Mo940 and noninoculated tissues grown under either high or low light intensity were colorless. The water extract from PM exhibited absorption maxima at 535, 505, and 335 nm whereas those extracts from other tissues only absorbed below 300 nm. Paper chromatography of the extract from corn line PM demonstrated the presence of visible bands of pink to purple color at R_f values of 0.29, 0.31, 0.36, 0.41, and 0.45. No such bands were detected in the water-soluble extracts from other tissues. The red pigments therefore were determined to be a mixture of anthocyanins by their ease of extraction and water solubility (8), chromatographic behavior (3), and absorption maxima (2). Individual anthocyanins were not identified.

Comparison of resistant, hypersensitive-resistant, and susceptible lines within each light treatment (Table 1) showed that lesion size (but not lesion type) often was similar under low light conditions. For example, lesion sizes on the resistant lines A632 and CI74 were not different from those on the susceptible hybrid, Deep Gold. Lesions on the hypersensitive-resistant lines PM and 33-16 were not different from lesions on the resistant line CI74 and the susceptible hybrid, Deep Gold.

TABLE 1. Effect of light intensity on corn anthracnose lesion size[a]

Corn line	Lesion[b] type	Lesion size (mm^2)[c] under high light (37,600 lux)	Lesion size (mm^2) under low light (9,600 lux)	Lesion size (mm^2), difference between high and low light[d]
Mo940	S	10.72 a	9.73 a	+0.99
Mo17	S	7.30 b	9.35 ab	−2.05
Deep Gold	S	4.98 c	5.57 cd	−0.59
K4	S	2.84 d	7.07 bc	−4.23*
CI74	R	1.95 d	5.44 cd	−3.49*
PM	HR	1.95 d	4.33 d	−2.38*
A632	R	1.85 d	7.46 abc	−5.61*
33-16	HR	1.43 d	3.88 d	−2.45*

[a]Lesions measured 8 days after inoculation with *Colletotrichum graminicola*.

[b]Symbols: S = susceptible; R = resistant; and HR = hypersensitive-resistant lesion type. Lesion type was not affected by light intensity.

[c]Values represent the mean size (mm^2) for 56 lesions per corn line (seven lesions for each of four plants replicated twice). Statistical analyses showed no variance among leaves for lesion size within any corn line under either light intensity. Numbers followed by the same letter in a column are not different according to Newman-Keuls sequential range test, $P = 0.05$.

[d]Differences in lesion size on each corn line under high and low light intensity. Numbers marked with asterisks indicate a significant change ($P = 0.02$) in lesion size due to high light intensity.

However, under high light, the susceptible lines Mo940, Mo17, and Deep Gold exhibited lesion sizes statistically different ($P = 0.05$) from all of the resistant and hypersensitive-resistant lines. Lesion size on inbred K4 was statistically different ($P = 0.05$) from other susceptible hosts, but not from resistant hosts. The resistant and hypersensitive-resistant lines all were classified in one lesion-size group (1.43 to 1.95 mm^2). Each of these hosts produced anthocyanin pigments around the lesions when plants were grown under high light intensity before and after inoculation. Under high light intensity the average lesion size on the susceptible inbred K4 was greatly decreased and the accumulation of anthocyanin pigments was associated with lesion development. In this respect, K4 reacted as a resistant line. However, the lesion type formed under both light intensities was of the susceptible type.

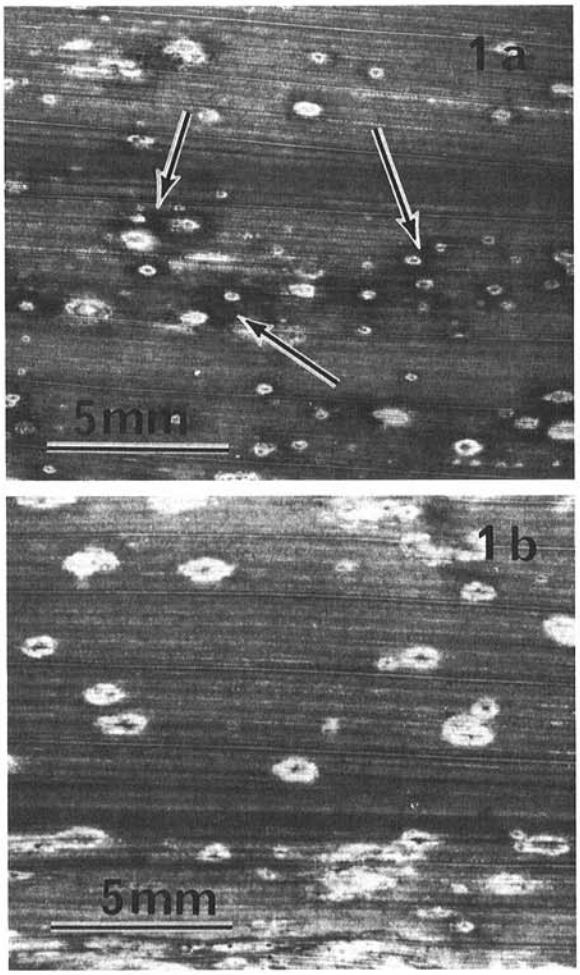

Fig. 1-(a, b). Resistant anthracnose lesion type on leaves of corn inbred CI74 at 8 days after inoculation with *Colletotrichum graminicola*. **a)** Plants grown under high light intensity (approximately 37,600 lux). Arrows indicate areas of anthocyanin accumulation surrounding lesions. **b)** Plants grown under low light intensity (approximately 9,600 lux). Note the lack of anthocyanin accumulation around lesions and the larger lesion size as compared to those in 1-a.

DISCUSSION

Lesion size alone was a poor criterion for classification of resistance or susceptibility of corn lines to *C. graminicola* (6). The present investigation demonstrates that increased light intensity significantly reduced lesion size on resistant and hypersensitive-resistant corn lines, but had no effect on lesion type. A similar reduction in lesion size without a change in lesion type was observed on the susceptible inbred K4. This suggests that potentially resistant corn lines may be overlooked on the basis of lesion size when screening for resistance under low light conditions (i.e., in the greenhouse under variable light intensities).

If host metabolism, which was not associated with the host-parasite interaction, had been the major factor in reducing lesion size, then a decrease in lesion size should have occurred on all corn lines. However, a statistically significant reduction in lesion size only occurred in lines which exhibited the accumulation of anthocyanins around lesions when plants were grown under high light intensity. The anthocyanins are glycosides of anthocyanidins and therefore represent the final products of flavonoid phenolic metabolism. The accumulation of anthocyanins suggests the possibility that phenolic metabolism may be involved in the resistance of corn to *C. graminicola*. It is known that light is required for anthocyanin and phenolic biosynthesis in corn (9, 10) and other grasses such as sorghum (8). However, the mechanism of light-induced phenolic metabolism in corn is not clear (9). Although corn inbred K4 produced a susceptible lesion type under both light intensities, lesion size was decreased significantly under high light intensity and anthocyanins accumulated around lesions. This suggests that light may induce similar changes in phenolic metabolism in some anthracnose-susceptible corn lines.

Other workers (7, 14) have reported qualitative observations indicating a decrease in anthracnose severity under high light irrespective of resistance or susceptibility. Our data show that lesion size was significantly reduced in some corn lines when plants were grown under high light. Thus, we propose that high light intensity enhanced the expression of host resistance through the effect of light on regulation of phenolic metabolism (8, 9, 10).

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