Light and Antifungal Polyacetylene Compounds in Relation to Resistance of Safflower to Phytophthora drechsleri

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ABSTRACT

Six-week-old greenhouse plants of the safflower cultivar Biggs were wound-inoculated in the first internode with a virulent isolate of *Phytophthora drechsleri*. Preinoculation treatments for 72 hr of either continuous light at 1,300 ft-c or continuous darkness immediately prior to inoculation had little effect on the postinoculation reaction. Plants held in controlled-environment rooms with 1,300 ft-c of light for 0, 8, 16, and 24 hr/24 hr period after inoculation were susceptible, moderately susceptible, moderately resistant, and resistant, respectively. Two antifungal polyacetylenes, safynol (*trans-trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol) and dehydrosafynol (*trans*-11-tridecene-3,5,7,9-tetrayne-1,2-diol) were extracted with methanol from infected stems 48 hr after inoculation. Subsequently, the compounds were isolated by thin-layer chromatography, using three successive solvent systems. After adjustment for loss during chromatography, infected stems at 0, 8, 16, and 24 hr light/24 hr period contained, respectively, 1,119, 2,233, 2,703, and 3,053 μg safynol, and 13, 94, 251, and 373 μg dehydrosafynol/100 g fresh infected stems. Internodes from adult Biggs plants, wound-inoculated in the first internode with *P. megasperma* var. *sojae* (avirulent to safflower) and held in the greenhouse for 4 days, contained 1,500 μg safynol and 750 μg dehydrosafynol/100 g fresh infected tissue. These plants were resistant to subsequent infection by *P. drechsleri* for 4 to 6 days when grown in continuous darkness.

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Additional key words: Carthamus tinctorius, disease resistance.

Under natural light in either the field or greenhouse, both seedling and adult plants of safflower, *Carthamus tinctorius* L. 'Biggs', are resistant to stem rot incited by *Phytophthora drechsleri* Tucker (9, 12, 13). However, when grown under low-intensity light, 3-week-old plants are susceptible (7, 9). Seasonal variation in lesion development around wound-inoculation sites indicates that light also affects the resistance of adult plants. Furthermore, in greenhouse studies, we observed that lesions were smaller on the sunny side than on the shaded side of adult Biggs plants wound-inoculated in the lower stem with *P. drechsleri*.

Two antifungal polyacetylene compounds, safynol (*trans-trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol) and dehydrosafynol (*trans*-11-tridecene-3,5,7,9-tetrayne-1,2-diol), accumulate in the first internodes of safflower plants which have been wound-inoculated with *P. drechsleri* (1, 2, 3, 11). Adult Biggs plants produce about 300 μg dehydrosafynol and 3,370 μg safynol/100 g fresh infected stems 48 hr after inoculation (1). These concentrations are sufficient to account for cessation of lesion development.

This study determined the effect of light on resistance of adult Biggs plants, and related resistance to concentrations of safynol and dehydrosafynol which accumulated in Phytophthora-infected tissue. This information will be useful for assessing the role of the polyacetylenes in disease resistance.

MATERIALS AND METHODS.—Plants were grown and inoculum was prepared as described (10). Briefly, safflower plants of the cultivar Biggs were grown in steamed soil contained in porous 20-cm clay pots in the greenhouse. *Phytophthora drechsleri*, our isolate 201, was cultured at 27 C on lima bean agar in petri dishes. Six weeks after emergence, plants were wound-inoculated in the first internode. In disease resistance evaluations, a 7-mm incision was made halfway through the stem. For polyacetylene determinations, plants were wounded with pin pricks in four vertical rows, four/row and 3 mm apart, equally spaced around the stem. The wounded areas were covered with strips of inoculum (lima bean agar plate cultures) held in place with a strip of aluminum foil lined with plastic film. Control plants were wounded but not inoculated.

To determine the relationship of preinoculation light treatment to disease reaction, paired groups of plants were held in either total darkness or in continuous 1,300 ft-c of light (cool-white fluorescent supplemented by incandescent light) for 72 hr at 30 C. After inoculation, the plants were placed in the greenhouse. In postinoculation light tests, plants growing at 30 C were exposed to 1,300 ft-c of light for 0, 8, 16, and 24 hr/24-hr period.

The concentrations of safynol and dehydrosafynol were determined 48 hr after inoculation. Cross sections of stems (ca. 2 cm long) were sampled from infected and healthy plants from ca. 5 mm above and below the pin pricks. The sections were frozen with liquid nitrogen, lyophilized, and extracted 4 times with methanol, 3 ml/g fresh stems, in a blender for 5 min. Tissue fragments were recovered by filtration after each extraction. The combined alcohol extracts were reduced to an alcohol-free aqueous solution by distillation in vacuo at 28 C. The aqueous solutions

were extracted with peroxide-free diethyl ether and evaporated to near dryness in vacuo at 20 C. The resulting near dry samples under N_2 gas were dissolved in 3-5 ml anhydrous diethyl ether and applied to silica gel layers containing 2.5% fluorescent indicator. The compounds were chromatographed with benzene:ethyl acetate:formic acid, 75:24:1, v/v. Both safynol and dehydrosafynol were eluted from a band at R_f .18 to .38. These compounds, which quenched the fluorescence of the indicator under an ultraviolet lamp (254 nm), were chromatographed with chloroform:acetone:formic acid, 95:4:1, v/v. The compounds were eluted from a band at R_f .12 to .22, and were subsequently chromatographed with ethyl ether:petroleum ether:formic acid, 80:19:1, v/v. Safynol was eluted from a band at R_f .50 and dehydrosafynol was eluted from a band at R_f .65. The concentrations of safynol and dehydrosafynol in absolute ethanol were determined by optical density at 269 nm (ϵ = 61,600) and 269.7 nm (ϵ = 160,200), respectively. Due to the photosensitivity of the compounds, all operations were performed in the dark, or with as little light as necessary.

The dry weight of each healthy sample was recorded as a percentage of the fresh weight. For each infected sample, the fresh weight before inoculation (adjusted fresh weight) was calculated: adjusted fresh weight of infected sample = (grams dry weight of infected sample/grams dry tissue for 100 g fresh healthy stems) x 100.

The effect of inoculation of Biggs plants with *P. megasperma* var. *sojae* (avirulent to safflower) on subsequent infection by *P. drechsleri* was determined. The epidermis on one side of the first internode was scraped lightly with a knife blade. This wounded area was covered with a strip of inoculum (lima bean agar plate culture) which was held in place with plastic film and aluminum foil. Controls were wounded, but not inoculated. After holding the plants in the greenhouse for 4 days after inoculation, the original inoculum was removed and replaced with an 8-mm disc of inoculum of *P. drechsleri*. Then the plants were transferred from the greenhouse to a dark room and held at 30 C for 7 days. Safynol and dehydrosafynol concentrations in the wound-inoculated tissue were determined at the time of inoculation with *P. drechsleri*.

RESULTS.—*Disease reaction.*—Preinoculation light treatment had little or no effect on the disease reaction. The necrotic lesions that developed around wounds of plants held in total darkness prior to inoculation did not differ measurably in size or rate of development from those on plants held in continuous light.

Plants that received continuous light (1,300 ft-c) after inoculation were resistant. The only macroscopic reaction was the development of a necrotic lesion around the inoculated wounds. Four days after inoculation, lesions extended 1 mm or less from the wound. Subsequently, there was no enlargement of the lesions. More extensive lesion development occurred on plants that received 1,300 ft-c of light for 16 hr/24-hr period. Four days after inoculation, the necrotic lesions extended upward 18 mm from the wound. No wilting or tissue collapse occurred. We classified these plants as moderately resistant. Lesions on plants that received light for 8 hr/24-hr period had an average length of 52 mm, measured upward from the wound, 4 days after inoculation. Although the stems did not collapse, all plants wilted. Plants that received no light following inoculation were severely wilted 4 days after inoculation. Most of the stems collapsed, and lesions extended upward from the wound for an average distance of 150 mm.

Safynol and dehydrosafynol content of infected stems in relation to photoperiod.—The content of both safynol and dehydrosafynol varied directly with the photoperiod after inoculation. Forty-eight hr after inoculation with *P. drechsleri*, infected stems of plants held in continuous darkness contained 1,119 μg of safynol and 13 μg of dehydrosafynol/100 g adjusted fresh wt. Under continuous light, the tissue contained 3,053 μg safynol and 373 μg dehydrosafynol/100 g. Intermediate concentrations of the compounds were found under light treatments of 8 and 16 hr/24 hr. An analysis of variance of the data showed that the dehydrosafynol contents at each of the four treatments were significantly different at the .05 level (Table 1). Contents of safynol for light treatments of 0 hr vs. 8, 16, or 24 hr, and 8 vs. 24 hr, were significantly different.

The loss in weight of the stem sections after 48 hr of pin-prick inoculation for treatments of 0 hr, 8 hr, 16 hr, and 24 hr of light/24 hr were, respectively, 22, 22, 15, and 12%.

Effect of previously accumulated safynol and dehydrosafynol on the subsequent disease reaction in the dark.—Both safynol and dehydrosafynol accumu-

TABLE 1. Effect of postinoculation light treatment on the fresh weight content of safynol[a] and dehydrosafynol[b] in Biggs safflower stems 48 hr after wound-inoculation with *Phytophthora drechsleri*, and on the disease reaction

Light treatment[c]	Safynol[d]	Dehydrosafynol[d]	Disease reaction[e]
hr light/24 hr	*μg/100 g*	*μg/100 g*	
24	3,053a	373a	R
16	2,703ab	251b	MR
8	2,233b	94c	MS
0	1,119c	13d	S

[a] *Trans-trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol.

[b] *Trans*-11 -tridecene-3, 5,7,9-tetrayne-1,2-diol.

[c] Stems were wounded with 16 pin pricks, inoculated, and held at 30 C. Light (1,300 ft-c) and dark periods were alternated for a particular treatment, light period first.

[d] Average of three replications of 18 plants each. Means with the same letter are not different at the .05 level of significance. Content for 100 g adjusted infected fresh wt which was calculated from the dry weight of infected and uninfected fresh stems and represents the weight of infected stems, providing that no weight was lost during infection.

[e] Stems were wounded with a single 7-mm incision, inoculated, and held at 30 C for 96 hr. R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

lated in Biggs stems wound-inoculated with *P. megasperma* var. *sojae*. The affected stems of plants held in the greenhouse 4 days after inoculation with this fungus contained 1,500 μg safynol and 725 μg dehydrosafynol/100 g fresh infected tissue. Similar stem sections from healthy controls contained 83 μg safynol and less than 0.2 μg dehydrosafynol. Controls, which were wounded but not inoculated with *P. megasperma* var. *sojae*, were susceptible to *P. drechsleri* when held in continuous darkness. Lesions developed within 24 hr, and expanded rapidly thereafter. Production of lesions by *P. drechsleri* during continuous darkness in stems previously inoculated with *P. megasperma* var. *sojae* in the greenhouse was delayed for 4 to 6 days in ca. 88% of the plants. Infection probably did not occur in the other 12% of the plants, as lesions did not develop in 7 days. We were unable to reisolate the fungus from plants on which no lesions developed.

DISCUSSION.–Under natural light in the greenhouse, wound-inoculated first internode stems of the Biggs cultivar of safflower are resistant to *P. drechsleri* (11). There is sufficient accumulation of safynol and dehydrosafynol 48 hr after inoculation to account for cessation of lesion expansion. These results indicate that the compounds may play a role in limiting infection in a resistant reaction. Results of the light duration experiments reported here provide evidence that the concentrations of these compounds may determine the type of reaction.

Level of resistance, as affected by postinoculation light period, was related to concentration of the two antifungal polyacetylene compounds. Resistant reactions were obtained with postinoculation light treatments of 16 and 24 hr/24 hr. Sufficient safynol, ED_{50}=12 μg/ml (10), and dehydrosafynol, ED_{50}=1.7 μg/ml (1), accumulated in 48 hr in these light treatments to account for cessation of lesion development. In treatments of either 0 or 8 hr light/24 hr, the plants were susceptible to *P. drechsleri*, and the concentration of safynol and dehydrosafynol were too low to limit growth of the fungus.

The reaction of Biggs in continuous darkness or 8 hr light/24 hr after inoculation indicates that the tissue can be rapidly invaded and colonized when antifungal compounds are present in low concentration. The delay in colonization in continuous darkness of Biggs tissue containing relatively high concentrations of the compounds, induced by *P. megasperma* var. *sojae*, also suggests that the concentration of the antifungal compounds determines the reaction.

The direct relationship of light duration and polyacetylene accumulation suggests that light may be affecting photosynthesis, which in turn determines the levels of photosynthetic products available for translocation to the infection site in the safflower stem. Johnson's shading experiments with Biggs safflower (6) indicated the leaves may play a role in the resistance of the hypocotyl to *P. drechsleri*. In sugarcane, the translocation of photosynthate occurs very rapidly in the light, but almost stops in the dark (5). Also, translocation decreases in the dark in sugar beet (4). Should translocation of photosynthate in safflower be as fast as in other plants (8, 14), and as dependent on light as in sugarcane and sugar beet, then the differential response of safflower stems to wound-inoculation by *P. drechsleri* in the greenhouse (for example, small lesions on sunny side vs. larger lesions on shaded side) and in the controlled-environment rooms could be directly related to the concentration and rate of translocation of photosynthate in the stem. The biosynthetic mechanisms for the rapid production of antifungal polyacetylenes may be sensitive to the availability of energy yielding compounds (photosynthate). These conclusions are consistent with our observation that only the available light after inoculation determines the disease reaction.

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