

Symptom Development and Resistance in Safflower Hypocotyls to *Phytophthora drechsleri*

Lowell B. Johnson

Assistant Research Plant Pathologist, Department of Plant Pathology, University of California, Davis. Present address: Department of Plant Pathology, Kansas State University, Manhattan 66502.

Supported through Cooperative Agreement, Crops Research Division, ARS, USDA, and the University of California, Davis.

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Accepted for publication 22 October 1969.

ABSTRACT

The hypersensitive response and number of dead plants of resistant Biggs safflower (*Carthamus tinctorius*) inoculated with *Phytophthora drechsleri* was greater under low than under high light intensities at 27 C. Hypocotyl cross sections, 24 hr after inoculation, showed no morphological basis for this difference under the two light intensities, although slightly more fungal mycelium was evident in the low-light plants. The preinoculation light effect on plant response and death was not modified by shifting plants from high to low light or vice versa or to darkness for up to 48 hr during and after inoculation. Longer exposures (3-5 days) to darkness or the covering of plant tops with aluminum foil resulted in extensive killing of inoculated Biggs. Excised Biggs leaves were capable of a resistant reaction. Terminal grafts of Biggs tops on Nebraska 10 (N10, susceptible) hypocotyls and roots and N10 tops on Biggs roots and hypocotyls showed that hypocotyl resistance or susceptibility was unchanged regardless of the reaction of the top.

Hypocotyls of uprooted Biggs and N10 plants showed typical symptoms following a 24-hr inocula-

tion and flooding period when compared to plants inoculated directly in crocks, as did plants with all roots removed. However, only a portion of those plants that were uprooted, and which had their tops excised at the cotyledonary node, showed any hypocotyl symptoms when flooded during and after inoculation.

Hypocotyls of Biggs and N10, excised after a 2-hr inoculation period and immersed in 15 ml water in test tubes, showed no symptom development, although hypocotyls of uprooted plants in the same test tubes did. Symptoms developed if hypocotyls were instead plated on moist filter paper in petri plates.

Material(s) showing a pink coloration and blue ultraviolet fluorescence leached through the hypocotyl epidermis when excised inoculated hypocotyls were wrapped in moist chromatography paper. This leaching was more intense in inoculated Biggs than in inoculated N10. Its relationship to resistance and symptom development was not determined. Phytopathology 60:534-537.

Resistance in the safflower (*Carthamus tinctorius* L.) breeding line Biggs to root rot caused by *Phytophthora drechsleri* Tucker is modified by both temperature (4) and light (4, 6). Increased temperatures (up to 27 C) result in intensified hypocotyl reactions to the pathogen; considerable plant death occurs in a 27 C low-light environment. At least a portion of the temperature effect is directly on the host. The pathogen is favored in vitro by high temperatures (27 to 30 C), and might also be more pathogenic under higher temperatures (4).

Little is known concerning the mechanism by which light influences the resistant reaction in safflower. Hypocotyl contents of water-insoluble calcium and pectic substances appear related to resistance on a fresh wt basis but not on a dry wt basis, considering plants from several light environments. Other mechanisms whereby light influences resistance may be involved (6). This paper reports a further definition of the role of light and leaves in the resistant Biggs reaction, as well as other observations on the nature of this resistance.

MATERIALS AND METHODS.—Resistant Biggs and susceptible Nebraska 10 (N10) plants were grown in 0.95-liter crocks, with 7-8 plants/crock, as described previously (5).

Plants were grown either in a greenhouse or in growth chambers, as specified. Temperature and light were

measured as in earlier work (4). All growth chamber plants were subject to a 14-hr light period and a 10-hr dark period.

Zoospores of *P. drechsleri* (isolate 201) were obtained from lima bean agar-glucose plates flooded with soil extract (4). Plants 3 to 4 weeks old were flooded and inoculated, generally with 1.0 to 1.5×10^5 zoospores/crock. Crocks were drained 22-24 hr after inoculation.

Excised first or second leaves were inoculated in petri plates kept humid with moist filter paper. Water drops (usually 0.05 ml/drop) containing 50 to 2,500 zoospores were placed on the upper leaf surface at two to four locations along the leaf. Plates were left in the laboratory at approximately 21 C for leaf symptom development.

Plants for hypocotyl sectioning, or hypocotyl sections, were generally inoculated with 1.0×10^4 zoospores/ml suspensions for 1-4 hr. Hypocotyls were excised from plants, either before or after inoculation, and transferred either to petri plates with moist filter paper or to zoospore-free solutions or water. In some experiments, hypocotyls were wrapped in approximately 4-cm² sections of Whatman No. 1 chromatographic paper after inoculation and placed in moist petri plates. Symptom development was generally evaluated 24 hr after inoculation.

Hypocotyl epidermal strips and leaves were stained for location of the fungus with cotton blue-lactophenol. Tissue sections were fixed and stained as in earlier work (5).

Four-week-old plants were used for grafting experiments. Terminal grafts were made by inserting a V-shaped tongue cut on the base of the cotyledonary node into a notch cut in the hypocotyl receiving the top. Wounds were covered with petroleum jelly. The two sections were held in place by a spring-type clothespin. Grafted plants were maintained in moist plastic bags for at least 1 week in low light, and given at least 2 weeks to recover from the grafting shock before inoculation.

RESULTS.—The Biggs hypersensitive response under low (910 to 1,280 ft-c) and under high (2,340 to 3,440 ft-c) light intensities at 27 C was studied in hypocotyl cross-sections. Previous work had shown a massive hypersensitive response and considerable plant death in a low-light environment at 27 C (4). No morphological basis could be detected for differences in resistance of plants grown in the two light intensities. However, the extent of cell necrosis at infection sites was greater in plants from the low-light environment; and slightly more fungal mycelium was evident, although still difficult to detect.

To characterize the time of light influence, plants grown at 27 C under 3,440 ft-c of light were transferred to a low-light (1,280 ft-c) environment during a 24-hr inoculation period with 1.0×10^5 zoospores/crock, then returned to their original environment to permit any further development of symptoms. A comparable set of plants grown under the low-light environment was transferred to high light during inoculation and then returned. Controls in both environments were not moved during inoculation. No detectable change in reaction intensity could be observed as a result of the light change during inoculation for either set of plants, as compared to unmoved controls. In a second experiment, plants from the high-light environment at 27 C were placed in the dark at 27 C for either the 24-hr inoculation period or for 48 hr before and during inoculation. Again, no obvious difference was noted in reaction intensity when compared to controls in the high-light environment.

Biggs resistance in plants from a high-light environment could be overcome, however, by longer exposures to darkness. Three days of darkness before and during inoculation resulted in death of 8 of 19 plants, and the number of dead plants was even greater in longer periods of postinoculation darkness (Table 1). By the time of inoculation, plants showed increased rankness of growth and some leaf chlorosis from the 2-day pre-treatment in the dark.

These data suggest that the initial light effect is on the host rather than on the pathogen. This was further tested by covering Biggs leaves with aluminum foil to exclude light during and after inoculation while leaving the hypocotyl uncovered. In two greenhouse experiments, one with stab-inoculated hypocotyls and one with zoospore-inoculated uninjured hypocotyls, hypocotyls became completely necrotic in cross section

TABLE 1. Effect of exposure to darkness on killing of Biggs safflower plants by *Phytophthora drechsleri*

| Plant treatment ^a | No. plants killed/no. inoculated ^b |
|------------------------------|---|
| Standard light | 3/15 |
| 3 days darkness | 8/19 |
| 4 days darkness | 12/18 |
| 5 days darkness | 16/18 |

^a All environments at 30 C. The plants in the standard light treatment received a 14-hr light period of 2,500 ft-c and a 10-hr dark period. All others were grown in this environment until transfer to the dark. The 3-day darkness treatment included 48 hr of darkness prior to inoculation and the 24-hr inoculation period. The 4- and 5-day darkness treatments also included 1 and 2 days postinoculation darkness, respectively.

^b Plant kill determined 7 days after inoculation. Zoospore concentrations were 1.0×10^5 zoospores/crock.

within 2-3 days when leaves were covered with foil. Comparably inoculated plants without foil-covered leaves exhibited typical resistance. A role of leaves in Biggs resistance was indicated.

The reaction of Biggs and N10 leaves to *P. drechsleri* infection was studied in several experiments in which excised leaves in moist petri plates were used. Biggs leaves showed a hypersensitive fleck to zoospore inoculations. Leaves maintained their resistance for 3 days or more under extremely low-light conditions (70 ft-c); but eventually symptoms began to develop resembling those of N10. Leaves of N10 showed a progressive development of dark, water-soaked lesions around inoculation sites, which were first apparent between 24 and 48 hr after the start of inoculation. Both lamina and midrib darkened, and eventually the whole leaf became necrotic. Cotton blue-lactophenol staining showed a progressive spread of the mycelium through N10 leaves, while mycelium was difficult to find during the resistant stage of Biggs leaves.

Since Biggs leaves are capable of a resistant response while N10 leaves are not, it is possible that some inhibitory material moving directly from the leaves is responsible for hypocotyl resistance. This was tested by grafting N10 tops on Biggs hypocotyls and roots, and Biggs tops on N10 hypocotyls and roots. In two greenhouse experiments totaling 8 to 10 plants of each graft combination, hypocotyls retained their typical reactions, regardless of the plant top, when inoculated with 1.0×10^5 zoospores/crock. Biggs grafted on Biggs and N10 grafted on N10 also showed typical reactions of ungrafted plants after inoculation.

The possible role of leaves in resistance and symptom development was further studied through numerous excised hypocotyl experiments. Biggs hypocotyls from greenhouse-grown plants with roots and leaves excised after an initial 2-hr hypocotyl inoculation period were placed in 15 ml deionized water in test tubes (two hypocotyls/tube) and left for 24 hr. No flecking reaction developed, while similarly inoculated hypocotyls from uprooted plants (pulled from the soil, with the top and 3-4 cm of the main root and several lateral roots intact), placed in the water in the same test tubes with the excised hypocotyls, showed the typical hypersensitive fleck. Biggs plants inoculated in a similar fashion,

but with tops intact and the roots removed at the hypocotyl base, showed typical hypocotyl symptoms, while development of symptoms on hypocotyls of uprooted plants with the tops removed at the cotyledonary node was erratic. Some hypocotyls showed typical symptoms while other hypocotyls showed none.

When Biggs hypocotyls were placed in petri plates on moist filter paper instead of in deionized water after inoculation and excision, the typical fleck reaction occurred approximately 6 hr after removal from the zoospore suspension. Excised Biggs hypocotyls immersed in water for 10 hr after inoculation, then placed in petri plates on moist filter paper, developed the fleck reaction, although flecks were not present when hypocotyls were first removed from the water. In a single experiment, none of several inoculated hypocotyls, immersed for 24 hr before being placed in petri plates, developed flecking. Necrotic flecking developed up to 6 hr faster on excised Biggs hypocotyls placed in petri plates on filter paper after inoculation, than on comparable uprooted Biggs plants, inoculated at the same time and maintained with their hypocotyls in water after the 2-hr inoculation period with zoospores.

Although symptoms did not develop on excised Biggs hypocotyls immersed in water, fungal growth was not prevented. Staining of hypocotyl strips with cotton blue-lactophenol showed considerable growth of the fungus on the surface and through the tissues in amounts much greater than in resistant-reacting hypocotyls.

Susceptible N10 hypocotyls inoculated and excised as in previous experiments were also tested for symptom development, both in moist petri plates and immersed in water in test tubes. In petri plates on moist filter paper, the typical light-brown discoloration of susceptible hypocotyls developed; and hypocotyl collapse often occurred after several days. As with Biggs, no symptoms developed in immersed hypocotyls, although fungal growth was present throughout the tissue.

The possibility that oxygen was required for symptom development by excised Biggs hypocotyls in water was tested by bubbling air into tubes containing hypocotyls excised 2 hr after inoculation. In two experiments, no symptoms developed.

Leaching out of substances might also explain the failure of excised immersed hypocotyls to develop symptoms. To test the possibility by replacement, hypocotyls were placed in the following solutions after inoculation: 0.001, 0.01, and 0.1 M glucose; 0.001, 0.01, and 0.1 M phenylalanine; 0.00025, 0.0025, and 0.025 M cinnamic acid; a mixture of 0.01 M phenylalanine and 0.0025 M cinnamic acid; 0.001 M chlorogenic acid; and water extracts from grinding healthy leaves (1 ml water/g fresh wt, undiluted, 0.1 dilution, and 0.01 dilution) or healthy hypocotyls (3 ml water/g fresh wt, undiluted, 0.1 dilution, and 0.01 dilution). None of these solutions resulted in symptom development. Only 0.025 M cinnamic acid was phytotoxic and fungitoxic. Other solutions, except for 0.1 M phenylalanine, stimulated visible growth of the fungus on the hypocotyl surface over that on the water controls. Sealing of the

cut hypocotyl ends with petroleum jelly failed to prevent loss of symptom development.

Materials leaching through the hypocotyl epidermis and into the water were demonstrated by wrapping excised hypocotyls in moist Whatman No. 1 paper after inoculation and placing them in moist petri plates. A pink coloration appeared in the paper enclosing the inoculated Biggs hypocotyls, often within 12 hr after inoculation. The hypocotyls developed the typical necrotic fleck reaction. The pink coloration appeared to reach a maximum around 24 hr, then sometimes faded. By contrast, paper surrounding healthy Biggs or N10 hypocotyls rarely showed more than traces of pink coloration. Papers surrounding inoculated N10 hypocotyls sometimes showed a paler coloration, but this appeared to develop more slowly. For both Biggs and N10 this coloration was more pronounced near the cut ends of the hypocotyls. However, hypocotyl injury was not essential for its appearance, since paper surrounding inoculated hypocotyls of uprooted Biggs plants showed a similar pink appearance when plants were maintained in moist chambers. This appearance was less pronounced with inoculated N10; and it was either weak or nonexistent with healthy hypocotyls of intact plants of both. Preliminary attempts to characterize the pink compound (or compounds), which also showed a blue fluorescence under both long (366 m μ) and short (254 m μ) ultraviolet lamps, were unsuccessful.

DISCUSSION.—The role of light in maintaining hypocotyl resistance appears to be initiated in the plant top. Biggs leaves show resistance, yet Biggs tops do not transmit their resistance when grafted to susceptible hypocotyls. Biggs hypocotyls are also not dependent on materials from resistant leaves to exhibit their resistance. These data suggest that the material(s) provided by leaves are not specific, and that they may be metabolite(s) normally translocated from the tops of safflower plants. Since the basal portions of the hypocotyls have little chlorophyll, it is possible that photosynthetic products are required.

Growth of Biggs under low light, removal of Biggs plants from high light to darkness for several days, growth of Biggs in 27 C (4), and treatment of several other resistant varieties with gibberellic acid (8) all result in increased hypocotyl elongation and in increased susceptibility of safflower to *P. drechsleri*. Since all these treatments alter many aspects of plant metabolism in addition to cell elongation, it is difficult to point to a single change which might explain the loss of resistance. It is possible that any or all of the above treatments may be influencing different components of the plant's total resistance mechanism.

Biggs hypocotyls capable of a hypersensitive response to *P. drechsleri* were not necessarily resistant. Growth and inoculation of safflower in a 27-C low-light environment resulted in an increased hypersensitive response, yet a greater number of plants were killed, as compared to plants grown and inoculated in either higher light or lower temperature environments. At least part of this increased hypersensitive response was due to the 27 C preinoculation temperature (4), and probably, to

the preinoculation light environment. The rate of development of hypersensitive necrosis and the rate of growth of the fungus in the tissues are not correlated in either wheat resistant to *Puccinia graminis* f. sp. *tritici* (1) or in potato resistant to *Phytophthora infestans* (7). It would also appear that the necrotic reaction in safflower does not by itself explain resistance.

In a study of the development of the hypersensitive response using excised hypocotyls, results were consistent with the hypothesis that some unidentified material(s) leaching from hypocotyls was necessary for the hypersensitive response, although the possibility of an additional requirement for oxygen was not ruled out. The fact that excised hypocotyls, removed from water shortly after inoculation, developed a hypersensitive response faster than did inoculated hypocotyls on uprooted plants maintained in water, suggests that this leaching process could also occur from intact plants and delay symptom development and also resistance. Again, a possible benefit from aerobic conditions cannot be eliminated. Hypocotyls of uprooted plants wrapped in filter paper showed materials to exude from uninjured inoculated hypocotyls. Increased flooding time after infection of intact plants results in a greater percentage of plants killed (4, 9) and could be related to this leaching phenomenon, since more than just an increase in infecting zoospores is involved (4).

The appearance of one or more compounds in filter paper enclosing safflower hypocotyls, following inoculation with *P. drechsleri*, needs further investigation. Its relationship to phytoalexin production (2) or increased permeability (3) is uncertain. However, it is apparent

that following infection an increased exit of one or more substances can occur through the epidermis and into an aqueous environment.

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