

Similarities Between Age-Related and Race-Specific Resistance of Soybean Hypocotyls to *Phytophthora megasperma* var. *sojae*

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ABSTRACT

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The youngest tissue at the tops of intact 6-day-old soybean (*Glycine max* 'Altona') hypocotyls displays typical race-specific responses to inoculation with zoospores of *Phytophthora megasperma* Drechs. var. *sojae* race 6 (compatible) and race 4 (incompatible). However, the tissue becomes increasingly resistant as it matures so that sites at the bottom of the hypocotyl are incompatible to both races. Incompatibility in all race and site combinations was overcome temporarily by heat treatment of the hypocotyl at 42.5 C or preinoculation wiping of the hypocotyl surface with a cotton swab soaked with organic solvents. Duration of the heat treatment (20, 40, or 60 min) needed to overcome resistance was proportional to the incompatibility of the race-site combination, suggesting that differences

were quantitative. Partial elimination of resistance frequently was accompanied by increases in necrosis and glyceollin production, presumably due to more extensive tissue colonization. Glyceollin production correlated closely with necrosis. Sensitivity to an elicitor preparation from *Pms* culture filtrate and to localized freezing injury, as measured by glyceollin production and necrosis, was greater in mature tissue. Our results indicate that race-specific and age-related resistance are basically similar, that differences between resistance and susceptibility are quantitative, and that the balance between the two generally favors resistance.

We reported previously that etiolated 6-day-old soybean (*Glycine max* (L.) Merr.) hypocotyls increase in resistance to *Phytophthora megasperma* Drechs. var. *sojae* Hildeb. (*Pms*) from the top to the bottom (17). Within a short distance on the hypocotyl surface, corresponding to a growth period of only 1-2 days, the interaction with compatible races changes from compatible to incompatible. Paxton and Chamberlain (19) also reported that soybean plants increase in resistance with age, and suggested that resistance may be governed by different mechanisms in older tissue.

However, because the differences in age of the tissues they examined were much greater (7-21 days) than in our experiments, the two studies may not be comparable.

Several workers (1,16,22) have proposed that the basis for incompatibility in the race-specific *Pms*-soybean interaction is the stimulation of glyceollin production by race-specific elicitors or incompatibility factors produced by the fungus and that compatibility occurs when such materials are not produced. This proposal does not appear to accommodate the change from compatibility to incompatibility that occurs in the maturing hypocotyl unless it is assumed that different mechanisms of resistance are involved. Observations of symptoms, glyceollin

production (17), and spread of the pathogen in host tissue (21) suggest that race-specific incompatibility and the incompatibility that develops in maturing hypocotyl tissue to the previously compatible race are expressions of a basically similar response. In this paper we report further evidence for this. Treatment of hypocotyl tissues with heat or organic solvents, both of which have been reported to overcome incompatibility barriers (6,25), had similar effects on both kinds of incompatibility.

MATERIALS AND METHODS

Fungus. Isolates of races 4 and 6 of *Pms* were obtained from R. I. Buzzell, Research Station, Agriculture Canada, Harrow, Ont., and were grown routinely and for zoospore production on V-8 juice agar at 25 C in the dark. Procedures for zoospore production have been described previously (17,23). Zoospores in suspensions were counted with a haemocytometer and (unless otherwise indicated) adjusted to 1×10^5 /ml with sterile distilled water.

Plant. The soybean plants used in these experiments were of the cultivar Altona, which is susceptible to *Pms* race 6 and resistant to race 4. Seeds were supplied by R. I. Buzzell, and etiolated seedlings were grown as described previously (17,23).

Hypocotyl inoculation. Six-day-old etiolated seedlings were washed in running tap water, blotted dry, and placed horizontally in glass trays as described (17,23). Intact hypocotyls were inoculated by placing three or four separate 10- μ l droplets of zoospore suspension at the top (1.5–2.0 cm below the cotyledons), the middle, or the bottom (2 cm above the roots). When required, hypocotyls were wounded at the top, middle, or bottom by making longitudinal slits about 1 cm long with the sharpened tip of a flat spatula. Inoculum suspensions or elicitor solutions (10 μ l) were placed in the wounds. Inoculated plants were incubated in moist conditions in the dark at 25 C for 24 hr.

Symptom development and necrosis. Susceptible lesions spread rapidly and the hypocotyl became water-soaked without necrosis and browning, above and below the inoculation site. Resistant lesions usually were restricted to the area beneath the inoculum drop and became uniformly brown and necrotic. Two variants of these responses are also referred to in this paper. These are "very resistant" in which only brown necrotic flecks developed beneath the inoculum drop and "spreading necrosis" in which, following certain treatments, brown necrotic lesions spread beyond the area of the inoculum drop.

Glyceollin determination. Diffusate drops were removed from un wounded hypocotyls by suction. Inoculated or elicitor-treated segments plus 5 mm of healthy tissue at each end were cut from the hypocotyls, placed in test tubes together with diffusates, covered with 4 ml of 95% ethanol, and heated by immersion of the tubes in boiling water for 3 min. The sampled tissue was steeped overnight at 10 C in the dark, the ethanol was replaced with fresh ethanol, and steeping was continued for an additional 24 hr. The ethanol extracts and an additional 2 ml used for rinsing were combined and reduced to dryness at 40 C on a rotary evaporator. The residue was extracted with 3×0.5 ml of ethyl acetate, the extract was transferred to small vials, and the solvent was removed under N_2 at 35 C.

For thin-layer chromatography, the extract was redissolved in 100 μ l ethyl acetate and 25–50 μ l was applied to channels on Whatman LK6DF silica gel plates (250 μ m) and developed in benzene:methanol (95:8, v/v). Glyceollin (a mixture of isomers) was located by reference to a standard (kindly supplied by P. Albersheim, Department of Chemistry, University of Colorado, Fort Collins) and its fluorescence quenching in ultraviolet light. The silica gel bands containing the glyceollin were scraped from the plates and eluted in small glass columns with ethyl acetate. The ethyl acetate extracts were dried under N_2 at 35 C and redissolved in absolute ethanol. Glyceollin concentration was calculated from the absorbance (285 nm), after subtraction of the absorbance values obtained from extracts of corresponding control tissues, and the extinction coefficient ($\epsilon = 10,300$) as described by Ayers et al (2). Duplicate or triplicate samples of a minimum of five plants were used for each determination and experiments were performed two

or more times. Data are presented as micrograms of glyceollin per milligram dry weight of excised infected tissue or, in experiments with elicitor, as micrograms of glyceollin per hypocotyl segment, without correction for losses during extraction. Absorbance values of un wounded uninoculated hypocotyl extracts were uniformly low, corresponding to glyceollin levels of 0.2–0.5 μ g/mg tissue dry weight.

Dry weight of infected tissues. Diseased tissues in *Pms*-inoculated hypocotyls, as indicated by necrosis or light microscopic examination, were dissected from the hypocotyl segments following extraction, dried, and weighed. In incompatible interactions, the minimum excision was to a depth of 0.5–1.0 mm. When interactions were highly incompatible, the inclusion of uninfected tissues was unavoidable and, if glyceollin accumulated only in brown necrotic tissue, probably resulted in an underestimation of glyceollin concentrations.

Heat treatment. Seedlings were heated according to the techniques described by Chamberlain and Gerdemann (6) and Chamberlain (5). All but the cotyledons and the uppermost 1 cm of the hypocotyl were immersed in distilled water at 42.5 ± 0.5 C for 20, 40, or 60 min. The seedlings then were dried and placed in glass trays and inoculated as described. A second series was allowed to recover for 24 hr prior to inoculation.

Localized freezing injury. The procedure was adapted from that described by Rahe and Arnold (20). Instead of applying dry ice directly, however, a steel rod 3 mm in diameter was cooled in dry ice and the end was applied briefly to the hypocotyl surface, thus ensuring that equal areas of tissue were frozen. The plants were placed in trays, then four injuries were made at the top and four at the bottom of the hypocotyl as for inoculation. The resulting lesion was similar in size to that produced by zoospores in a 10- μ l drop of inoculum.

Organic solvent treatment. The top 2 cm and the bottom 2 cm of hypocotyls were wiped lightly with a cotton swab soaked in chloroform or ethyl acetate. The solvent was allowed to evaporate completely before the treated segments were inoculated.

Preparation of elicitor. The *Pms* elicitor was prepared from fungal culture filtrates by modification of the technique of Ayers et al (2). Filtrates from 10-day-old cultures of *Pms* R4, growing on asparagine medium were concentrated in vacuo at 40 C to 1/10 the original volume. High-molecular-weight materials were precipitated first in 80% methanol, then redissolved in water and reprecipitated in 90% acetone. The acetone precipitate was dialyzed overnight in distilled water and further purified by Sephadex G-25 gel filtration in 10 mM PO_4 buffer, pH 7.2. All elicitor activity was detected in the void volume fractions. The carbohydrate content of the pooled active fractions was determined by the method of Dubois et al (7). The antibiotic neomycin sulphate (50 μ g/ml) was added to elicitor preparations and water controls.

RESULTS

Effect of heat treatment on host response and glyceollin production. Unheated hypocotyls inoculated with the incompatible race 4 were typically resistant at the top with uniformly necrotic lesions and high levels of glyceollin, and even more resistant at the middle and the bottom where both necrosis and glyceollin production were reduced (Table 1). Unheated hypocotyls inoculated with the compatible race 6 were: susceptible, with water-soaked spreading lesions with low glyceollin levels at the top; resistant, with necrosis at the middle (where glyceollin production was much increased); and resistant with limited necrosis and lower glyceollin levels at the bottom. In agreement with previous reports (5,6), heat-treated plants became susceptible to the incompatible race of *Pms* but recovered their resistance within 24 hr (Table 1). Longer periods of heating were required to produce the same degree of susceptibility at the middle and the bottom of the hypocotyl as at the top. Thus, hypocotyls heated for 20 min were susceptible to race 4 at the top, but remained resistant with increased necrosis at the middle and the bottom. This increased necrosis was accompanied by increases in glyceollin levels, although, in general, where heat treatment resulted in

susceptibility, glyceollin production was greatly reduced. Resistance to the compatible race 6 at the middle and the bottom was partly removed by heating for 20 min, although, as with race 4, an increase in necrosis at the bottom of the hypocotyl was accompanied by an increase in glyceollin production. In both glyceollin production and reaction type, hypocotyls heated for 20 min and inoculated with race 6 resembled those heated for 40 min and inoculated with race 4. Hypocotyls heated for 40 min were uniformly susceptible to race 6 at all three inoculation sites, but 60 min was required for similar susceptibility to race 4.

Effect of chloroform or ethyl acetate treatment on infection and glyceollin production. The response of hypocotyls to both *Pms* races was greatly affected by wiping the surface with chloroform or ethyl acetate. Race 6 caused more extensive water-soaking at the tops of treated hypocotyls than of untreated hypocotyls. At the bottom, coalescing light-brown lesions were produced over the entire treated surface, but glyceollin levels were similar or slightly higher than in untreated tissues (Tables 2 and 3). Race 4 produced coalescing water-soaked lesions at the tops of the hypocotyls, and glyceollin levels were lower than those in untreated plants. At the

bottom, lesions were barely detectable in untreated hypocotyls, but were uniformly necrotic, frequently spreading at the edges, and produced higher levels of glyceollin in treated tissues. Uninoculated plants were slightly discolored by chloroform treatment and accumulated small amounts of glyceollin. Reactions were normal when inoculation was delayed for 24 hr following treatment, which suggests that neither chloroform nor ethyl acetate caused permanent damage to the hypocotyls.

Effect of wounding and inoculum level on infection and glyceollin production. Disease development in wounded or unwounded hypocotyls was observed for 5 days. When inoculated with the compatible race 6 at the top or the middle, wounded hypocotyls became severely diseased. They developed spreading necrotic lesions when inoculated at the bottom. Intact hypocotyls were completely susceptible only when inoculated at the top. Limited, lightly necrotic, resistant lesions developed following inoculation at the bottom and the plants remained healthy.

Glyceollin levels in hypocotyls wound-inoculated with the compatible race 6 were consistently higher at the bottom of the hypocotyl than at the other two inoculation sites (Fig. 1). Increases

TABLE 1. The effect of heat treatment on host response and glyceollin production in soybean hypocotyls (cultivar Altona) inoculated with *Phytophthora megasperma* var. *sojae* (*Pms*) race 6 (compatible) or race 4 (incompatible) at the top, middle and bottom

Time of ^a inoculation after heating	<i>Pms</i> race	Site ^b inoculated	Host response and μg glyceollin ^c per mg infected tissue (dry weight) ^d after heat treatment for:			
			0 min	20 min	40 min	60 min
Immediately	6	T	S ^e 2.1	S 1.4	S 0.6	S 0.6
		M	SR 9.9	S 4.3	S 2.4	S 1.2
		B	R 2.8	SR 12.6	S 4.3	S 0.8
	4	T	R 20.1	S 7.2	S 0.8	S 0.4
		M	R 7.4	R 13.5	S 2.7	S 1.1
		B	VR 4.1	R 19.2	SR 8.5	S 0.6
After 24 hr	6	T		S 1.8	S 4.0	S 4.1
		M		SR 8.4	SR 11.1	S 5.9
		B		R 10.8	R 8.6	R 8.5
	4	T		R 22.8	R 16.9	R 19.2
		M		R 7.8	R 17.6	R 30.4
		B		VR 7.0	VR 10.5	VR 7.4

^a Etiolated 6-day-old seedlings were immersed to within 1 cm of the cotyledons in a water bath at 42.5 ± 0.5 C, removed after 20, 40, or 60 min, and inoculated either immediately or 24 hr later.

^b Three separate drops (10 μl) of a zoospore suspension (1×10^5 per milliliter) of *Phytophthora megasperma* var. *sojae*, race 4 or race 6 were used to inoculate sites on the hypocotyl at the (T) top, (M) middle, and (B) bottom. Inoculated segments of hypocotyl were harvested 24 hr after inoculation.

^c After subtraction of corresponding absorbance values ($\text{UV}_{285\text{nm}}$) in extracts from control tissues receiving similar treatment and water as inoculum.

^d Diseased tissue was excised from segments used for extraction and the dry weight determined.

^e S = susceptible; SR = spreading necrotic lesions; R = resistant, necrosis limited to area under inoculum drop; and VR = very resistant, slight flecking to no visible symptoms.

TABLE 2. Effect of chloroform on host response and glyceollin production in soybean hypocotyls (cultivar Altona) inoculated at the top or the bottom with *Phytophthora megasperma* var. *sojae* (*Pms*) race 6 (compatible) or race 4 (incompatible)

<i>Pms</i> race	Site inoculated	Host response and μg glyceollin per mg of infected tissue (dry weight) ^a	
		No chloroform	Chloroform ^b
Race 6	Top	S ^c 3.5 ± 2.4	S 1.0 ± 0.4
	Bottom	R 4.7 ± 2.2	SR 9.2 ± 3.4
Race 4	Top	R 22.2 ± 3.6	SR 7.6 ± 2.1
	Bottom	VR 9.7 ± 3.7	SR 30.0 ± 9.3

^a Hypocotyl segments were harvested 24 hr after inoculation. Absorbance values ($\text{UV}_{285\text{nm}}$) in extracts from similarly treated controls were subtracted from each value.

^b Top and bottom of the hypocotyls were wiped with chloroform-soaked absorbent cotton prior to inoculation with four separate drops (10 μl) of zoospore suspension (1×10^5 per milliliter).

^c S = susceptible; SR = spreading necrotic lesions; R = resistant, necrosis limited to area under inoculum drop; and VR = very resistant, slight flecking to no visible symptoms.

TABLE 3. Effect of ethyl acetate on host response and glyceollin production in soybean hypocotyls (cultivar Altona) inoculated at the top and bottom with *Phytophthora megasperma* var. *sojae* (*Pms*) race 6 (compatible) or race 4 (incompatible)

<i>Pms</i> race	Site inoculated	Host response and μg glyceollin per mg of infected tissue (dry weight) ^a	
		No ethyl acetate	Ethyl acetate ^b
Race 6	Top	S ^c 6.7 ± 0.3	S 6.8 ± 1.8
	Bottom	R 5.5 ± 2.1	SR 13.7 ± 0.6
Race 4	Top	R 35.5 ± 5.9	SR 22.5 ± 6.5
	Bottom	R 15.6 ± 5.1	SR 23.9 ± 5.4

^a Hypocotyl segments were harvested 24 hr after inoculation. Absorbance values ($\text{UV}_{285\text{nm}}$) in extracts from controls similarly treated were subtracted from each value.

^b Hypocotyls were wiped at the top and bottom with ethyl acetate-soaked absorbent cotton, prior to inoculation with four separate drops (10 μl) of zoospore suspension (1×10^5 per ml).

^c S = susceptible; SR = spreading necrotic lesions; R = resistant, necrosis limited to area beneath inoculum drop; and VR = very resistant, slight flecking to no visible symptoms.

in the inoculum concentration above 5×10^4 zoospores per milliliter did not cause any additional increase in glyceollin production. More glyceollin was produced following inoculation with race 4 than with race 6 and again highest concentrations tended to accumulate at the bottom of the hypocotyl and lowest at the top (Fig. 1). As with race 6, glyceollin accumulation was not correlated with inoculum concentration.

Effect of elicitor on glyceollin production. Wounded hypocotyls treated with elicitor at 0.25 μg glucose equivalents per site developed consistently higher levels of glyceollin at the bottom than at the top (Table 4). Such differences were demonstrable after only 8 hr and were maintained throughout 32 hr. The differences were eliminated by the application of 20 times more elicitor to the top of the hypocotyl than to the bottom (5.0 μg glucose equivalents at the top, 0.25 μg glucose equivalents at the bottom, Table 4). Wounding alone caused the accumulation of appreciable amounts of glyceollin at the bottom of the hypocotyl.

Effect of localized freezing on necrosis and glyceollin production. Very little necrosis developed at the top of the hypocotyl following freezing, but at the bottom the sites were uniformly necrotic and superficially appeared similar to *Pms*-inoculated hypocotyls. There was a wide difference in glyceollin production; six times as much accumulated at the bottom as at the top (Table 5).

DISCUSSION

Increases in disease resistance in older tissues have been described in soybeans (19) and other plants (eg, 3,4,8,13,15) and especially for seedling diseases caused by *Pythium* spp. (9). Garrett (12) considered many species of the Pythiaceae to be primitive parasites that cause diseases of immature tissues, but are unable to attack older tissues except when these have been predisposed by wounding or other stresses. Phytophthora rot of soybeans may be a disease of this type, for the most susceptible site in the hypocotyl is the young tissue immediately below the cotyledons. Under field conditions this region of the etiolated hypocotyl may be exposed to the pathogen in the soil for extensive periods prior to emergence, depending on temperature and moisture conditions.

The work reported here on the effect of tissue maturity on resistance of soybeans to *Pms* differs from that reported previously in two ways. The maturation range of the 6-day-old hypocotyl tissue used represented a growth period of only 2-3 days, and plants were inoculated by placing drops of zoospore suspensions on the hypocotyl surface, rather than by insertion of mycelium into hypocotyl wounds. Our results indicate that the response of wounded plants differs from that of intact plants. Thus, when hypocotyls were wound-inoculated at the bottom the compatible race 6 produced spreading necrotic lesions rather than restricted hypersensitive lesions, and there was little correlation between inoculum level and glyceollin production, contrary to previous findings with intact plants (17). Tissue-maturity related differences in reaction and glyceollin production were much reduced in

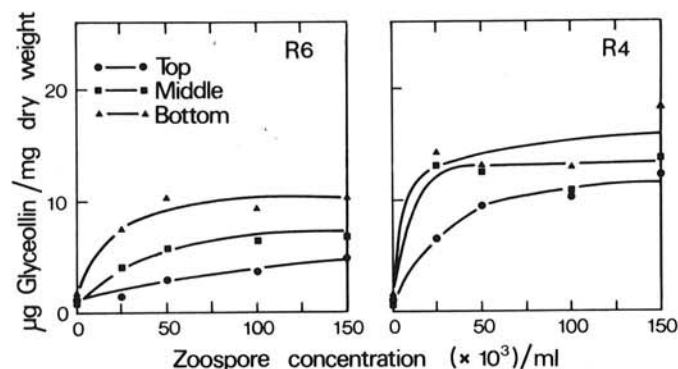


Fig. 1. Glyceollin accumulation (24 hr) following wound inoculation of soybean hypocotyls (cultivar Altona) at the top, middle, and bottom, with different concentrations of zoospores of *Phytophthora megasperma* var. *sojae* race 6 (compatible) and race 4 (incompatible).

wounded plants. Microscope observations indicate that epidermal cells are rarely penetrated (21); possibly they have a special influence on the course of the interaction in intact tissue. The production of more glyceollin at the bottom than at the top of the hypocotyl following wound inoculation or the application of elicitor to wounds, suggests that older tissue either has a greater ability to synthesize glyceollin or a greater sensitivity to factors that cause necrosis which may be necessary for glyceollin production. Two observations indicate that the second of these explanations is the most probable. These are: that much more necrosis developed at the bottom than at the top of the hypocotyl in response to freezing and that much more elicitor was required at the top than at the bottom for the production of similar amounts of glyceollin.

The effect of heat treatment in temporarily inhibiting both resistance and glyceollin production in heat-treated intact hypocotyls confirms the results obtained by Chamberlain and Gerdemann (6) with wound-inoculated tissues. The induction of susceptibility by wiping the hypocotyl surface with chloroform or ethyl acetate is similar to the effect of these and other organic solvents in overcoming stigma-pollen incompatibility (25) and again may be evidence of the importance of the epidermis in the normal interaction. The effects of the heat and solvent treatments were similar at all resistant-responding sites, both with the compatible as well as with the incompatible race. These results also support previous conclusions (17) that glyceollin production is correlated closely with tissue necrosis. The response to freezing injury provides additional evidence for this. Where heat and solvent treatments only partially removed resistance, increases in glyceollin production and necrosis occurred, presumably due to more extensive tissue colonization. While this suggests that glyceollin production may not be correlated closely with resistance,

TABLE 4. Effect of low and high concentrations of elicitor on the rate of glyceollin accumulation in wounds at the top and bottom of soybean hypocotyls (cultivar Altona)

Elicitor concentration ^a	Wound site ^b	Glyceollin (μg per hypocotyl) ^c after incubation for:			
		8 hr	14 hr	20 hr	32 hr
0.25	Top	0.6 \pm 0.1	0.9 \pm 0.4	5.3 \pm 2.3	9.0 \pm 0.7
	Bottom	2.2 \pm 0.4	4.8 \pm 1.1	8.5 \pm 0.3	21.0 \pm 3.0
5.00	Top	0.9 \pm 0.1	6.0 \pm 2.5	7.1 \pm 0.3	20.7 \pm 2.0
	Bottom	2.2 \pm 0.2	5.3 \pm 0.3	14.1 \pm 3.7	29.8 \pm 4.7
Buffer control	Top			0.8 \pm 0	0.5 \pm 0.1
	Bottom			3.1 \pm 0.7	5.5 \pm 1.5

^a Glucose equivalents (μg) per inoculation site. Elicitor was partially purified from *Pms* R4 culture filtrates by precipitation and Sephadex G-25 gel filtration.

^b Hypocotyl wounds were longitudinal slits 1 cm long either at the top (1-2 cm below the cotyledons) or the bottom (approximately 2 cm above the roots).

^c Means and standard errors were calculated from duplicate experiments.

TABLE 5. The effect of localized freezing on the accumulation of glyceollin at sites at the top and bottom of 6-day-old soybean hypocotyls (cultivar Altona)

Site treated ^a	Glyceollin production (μg per mg [dry wt] of necrotic tissue) during posttreatment incubation for:	
	24 hr	48 hr
Top	0.7	0.9
Bottom	4.7	5.4

^a A steel rod (3 mm in diameter), cooled in dry ice, was used to produce four symmetrical injury sites about 5 mm apart at the top and bottom of each hypocotyl.

^b The results are the average of replicated samples of 10 plants, corrected by subtraction of absorbance values ($\text{UV}_{285\text{nm}}$) obtained with similar extracts from uninjured controls.

^c Necrotic tissue was excised from hypocotyl segments and the dry weight determined.

alternatively it could be a reflection of the difficulty in excising only the infected tissue from very resistant lesions. Inclusion of uninfected tissue would lead to calculations of glyceollin levels that were artificially low.

Bateman and Lumsden (3) reported that increases in the resistance of *Phaseolus vulgaris* hypocotyls to *Rhizoctonia solani* were correlated with the conversion of pectin to pectate as tissues matured. English and Albersheim (10) and Nevins et al (18) reported that a number of other changes occur in cell wall chemistry that may be correlated with the development of resistance in maturing hypocotyls of the same host to *Colletotrichum lindemuthianum*. Presumably, changes in cell wall structure and chemistry also occur in developing soybean hypocotyls and arguments could be advanced that such changes account for the higher resistance to *Pms* at the bottom of the hypocotyl. However, they would not account for the resistance of immature tissue at the top of the hypocotyl to incompatible races of *Pms*. Neither the results reported here nor microscope observations of the interaction (21) suggest that the mechanisms involved are different. Thus, heat and solvent treatments eliminated or reduced the resistance of both mature and immature hypocotyl tissue. This suggests not only that the mechanisms of resistance are similar, but that changes in cell wall chemistry or structure, if they occur, are not critical for resistance, especially in view of the ability of the tissues to recover their normal reactions within 24 hr (Table 1). The effects of heat treatment also indicate that differences in resistance are quantitative rather than qualitative. The greater the incompatibility of the inoculum site-*Pms* race combination, the longer the heat exposure required to overcome its resistance. Furthermore, at the bottom of the hypocotyl, where both races are incompatible, distinctions between races persisted. Resistance to race 6 was eliminated by a shorter heat exposure than was required for race 4. Intrinsic differences in ability of the races to produce appropriate cell wall-degrading enzymes are unlikely to provide an explanation for this because the relative virulence of the two races is reversed on other cultivars (14,23). Therefore, it seems improbable that changes in cell wall chemistry or structure would account for the observed increases in resistance of maturing hypocotyl tissue.

Proposals (16,22) that specificity in the soybean-*Pms* interaction is due to the production of race-specific elicitors or incompatibility factors by incompatible races of *Pms* are difficult to reconcile with the results reported here. According to these proposals *Pms* race 6 would be compatible on Altona because it lacks an elicitor recognized by an Altona gene for resistance. Logically it should be compatible also in mature hypocotyl tissue, but it is not. If it is assumed that *Pms* race 6 produces a nonspecific elicitor that causes incompatibility in the more mature tissue, then it is equally difficult to explain why such an elicitor does not cause incompatibility when hypocotyls are inoculated at the top.

In conclusion, the results of these and earlier experiments (17,21) suggest that a balance exists between compatibility and incompatibility, similar to the concept outlined by Bell (4) for Verticillium wilt of cotton. Under most circumstances this favors incompatibility. Only in the immature tissue at the top of the hypocotyl is the pathogen able to tip the balance towards compatibility. This region also was the least responsive to freezing injury and to the elicitor extract preparation. The evidence suggests that incompatibility is a general response to nonself that can occur with either race. Compatibility may be specific ability to override this response in immature tissue, possibly mediated by factors that temporarily stabilize the host cell (24) or suppress the incompatible response (11).

LITERATURE CITED

1. Albersheim, P., and Valent, B. S. 1978. Host-pathogen interactions in plants. Plants, when exposed to oligosaccharides of fungal origin defend themselves by accumulating antibiotics. *J. Cell Biol.* 78:627-643.
2. Ayers, A. R., Ebel, J., Finelli, F., Berger, N., and Albersheim, P. 1976.

- Host-pathogen interactions. IX. Quantitative assays of elicitor activity and characterization of the elicitor present in the extracellular medium of cultures of *Phytophthora megasperma* var. *sojae*. *Plant Physiol.* 57:751-759.
3. Bateman, D. F., and Lumsden, R. D. 1965. Relation of calcium content and nature of pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhizoctonia solani*. *Phytopathology* 55:734-738.
4. Bell, A. A. 1969. Phytoalexin production and Verticillium wilt resistance in cotton. *Phytopathology* 59:1119-1127.
5. Chamberlain, D. W. 1970. Temperature ranges inducing susceptibility to *Phytophthora megasperma* var. *sojae* in resistant soybeans. *Phytopathology* 60:293-294.
6. Chamberlain, D. W., and Gerdemann, J. W. 1966. Heat-induced susceptibility of soybeans to *Phytophthora megasperma* var. *sojae*, *Phytophthora cactorum*, and *Helminthosporium sativum*. *Phytopathology* 55:70-73.
7. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
8. Elliston, J., Kuć, J., and Williams, E. B. 1976. A comparative study in the development of compatible, incompatible and induced incompatible interactions between *Colletotrichum* spp. and *Phaseolus vulgaris*. *Phytopathol. Z.* 87:289-303.
9. Endo, R. M., and Colt, W. M. 1974. Anatomy, cytology, and physiology of infection by *Pythium*. *Proc. Am. Phytopathol. Soc.* 1:215-223.
10. English, P. D., and Albersheim, P. 1969. Host-pathogen interactions: I. A correlation between α -galactosidase production and virulence. *Plant Physiol.* 46:217-224.
11. Garas, N. A., Doke, N., and Kuć, J. 1979. Suppression of the hypersensitive reaction in potato tubers by mycelial components from *Phytophthora infestans*. *Physiol. Plant Pathol.* 15:117-126.
12. Garrett, S. D. 1956. *Biology of root-infecting fungi*. Cambridge University Press, London. 294 pp.
13. Griffey, R. T., and Leach, J. G. 1965. The influence of age of tissue on the development of bean anthracnose lesions. *Phytopathology* 55:915-918.
14. Haas, J. H., and Buzzell, R. I. 1976. New races 5 and 6 of *Phytophthora megasperma* var. *sojae* and differential reactions of soybean cultivars for races 1 to 6. *Phytopathology* 66:1361-1362.
15. Hunter, R. E., Halloin, J. M., Veech, J. A., and Carter, W. W. 1978. Terpenoid accumulation in hypocotyls of cotton seedlings during aging and after infection by *Rhizoctonia solani*. *Phytopathology* 68:347-350.
16. Keen, N. T., and Bruegger, B. 1977. Phytoalexins and chemicals that elicit their production in plants. Pages 1-26 in: P. A. Hedin, ed. *American Chemical Society Symposium Series 62*. American Chemical Society, Washington, DC. 286 pp.
17. Lazarovits, G., Stössel, P., and Ward, E. W. B. 1981. Age-related changes in specificity and glyceollin production in the hypocotyl reaction of soybeans to *Phytophthora megasperma* var. *sojae*. *Phytopathology* 71:94-97.
18. Nevins, D., English, P., and Albersheim, P. 1968. Changes in cell wall polysaccharides associated with growth. *Plant Physiol.* 43:914-922.
19. Paxton, J. D., and Chamberlain, D. W. 1969. Phytoalexin production and disease resistance in soybeans as affected by age. *Phytopathology* 59:775-777.
20. Rahe, J. E., and Arnold, R. M. 1975. Injury-related phaseollin accumulation in *Phaseolus vulgaris* and its implication with regard to specificity of host-parasite interaction. *Can. J. Bot.* 53:921-928.
21. Stössel, P., Lazarovits, G., and Ward, E. W. B. 1980. Penetration and growth of compatible and incompatible races of *Phytophthora megasperma* var. *sojae* in soybean hypocotyl tissues differing in age. *Can. J. Bot.* 58:2594-2601.
22. Wade, M., and Albersheim, P. 1979. Race specific molecules which protect soybeans from *Phytophthora megasperma* var. *sojae*. *Proc. Nat. Acad. Sci. USA* 76:4433-4437.
23. Ward, E. W. B., Lazarovits, G., Unwin, C. H., and Buzzell, R. I. 1979. Hypocotyl reactions and glyceollin production of soybean cultivars inoculated with zoospores of races of *Phytophthora megasperma* var. *sojae*. *Phytopathology* 69:951-955.
24. Ward, E. W. B., and Stoessl, A. 1976. On the question of 'elicitors' or 'inducers' in incompatible interactions between plants and fungal pathogens. *Phytopathology* 66:940-941.
25. Willing, R. R., and Pryor, L. N. 1976. Interspecific hybridisation in poplar. *Theoret. Appl. Genet.* 47:141-151.