Temperature Ranges Inducing Susceptibility to Phytophthora megasperma var. sojae in Resistant Soybeans

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ABSTRACT

Recovery of resistance to *Phytophthora megasperma* var. *sojae* in heat-treated Harosoy 63 soybean plants began between 12 and 16 hr after treatment, and was completed between 20 and 24 hr after treatment. Loss of resistance was irreversible in plants treated for 2 min at 50 or 55 C. Heat-treated plants stored at 10 C did not recover resistance in 48 hr. Stem tissues 2 cm above the treated area were susceptible; tissues 3 cm above the treated area were resistant. Phytopathology 60:293-294.

Chamberlain & Gerdemann (1) reviewed the literature pertaining to loss of disease resistance through heat treatment in 1966. They demonstrated heat-induced loss of disease resistance and the inhibition of phytoalexin production by heat treatment of entire seedling soybean plants (1).

In the present investigation, I was concerned with some hitherto unexplored aspects of induced susceptibility to *Phytophthora megasperma* Drechs. var. *sojae* A. A. Hildeb. in soybean plants, the minimum time required for heat-treated plants to recover resistance, the effect of different temperature/time combinations on subsequent recovery of resistance, and the effect of heat treatment on stem tissues beyond the treated area.

Materials and Methods.—*Glycine max* (L.) Merr. 'Harosoy 63' and 'Harosoy' were used in this investigation. Harosoy 63 is highly resistant to *Phytophthora megasperma* var. *sojae*, and Harosoy is highly susceptible; otherwise the two varieties are indistinguishable. Soybeans were germinated and grown in washed builders' sand. Plants were heat-treated when they were 6-8 days old. Heat treatment, unless otherwise specified, consisted of immersing the roots and hypocotyls in a water bath (44 C) for 60 min. *Phytophthora* cultures were grown in a liquid medium made by autoclaving soybean seeds in distilled water (5-10 seeds/100 ml). Inoculations were made by inserting bits of mycelium in a wound made with a half-spear needle in the hypocotyl 2-3 cm below the cotyledonary node. The wounds were covered with petrolatum to prevent drying. Each treatment involved 10 or more plants. The results listed are averages of three or more tests for each individual treatment.

Results.—*Minimum period for recovery of resistance after heat treatment.*—Chamberlain & Gerdemann (1) noted that resistance in Harosoy 63 plants was recovered within 48 hr after heat treatment (44 C for 60 min). To determine the minimum time for recovery of resistance, I inoculated Harosoy 63 plants with *P. megasperma* var. *sojae* 4, 8, 12, 16, 18, 20, and 24 hr after heat treatment. Susceptible Harosoy plants (untreated) were inoculated at each time interval as a check on pathogenicity of the inoculum. All plants were incubated at 28 C and examined daily for 7 days. Plants with no spread of browning beyond the inoculation wound were termed resistant.

Plants inoculated 4, 8, and 12 hr after heat treatment did not recover. At 16 hr after treatment, 40% of the plants were resistant; at 18 hr, 70%; at 20 hr, 85%; and at 24 hr, 100% were resistant. Recovery of resistance evidently begins between 12 and 16 hr after heat treatment, and complete resistance is recovered between 20 and 24 hr after heat treatment.

Temperature/time combinations inducing reversible and irreversible loss of resistance.—The effect of shorter periods of exposure at different temperatures was explored. Since Jerome & Müller (2) found that treating bean pods above 44 C resulted in irreversible loss of resistance, I investigated this possibility for soybean plants. Harosoy 63 plants were treated in a series of temperature/time combinations (Table 1). Immediately after heat treatment, all plants were inoculated with *P. megasperma* var. *sojae;* duplicate series of plants subjected to the same heat treatment were inoculated 24 hr after heat treatment to determine whether induced susceptibility was reversible. All plants were incubated at 28 C.

All treatments except 0.25 min at 50 and 55 C induced susceptibility to *P. megasperma* var. *sojae* in Harosoy 63 plants (Table 1). At all temperature/time combinations up to 15 min at 45 C, all plants recovered resistance in 24 hr; at higher temperatures, however, many plants remained susceptible after 24 hr. To determine whether a longer period between heat treatment and inoculation would permit the recovery of resistance, several lots of Harosoy 63 plants were treated at 50 C for 2 min. The plants were stored at 28 C and inoculated 48, 72, and 96 hr after heat treatment. This treatment induced irreversible loss of resistance (Table 1). At temperatures above 45 C, time intervals longer than those listed were lethal to the plants; consequently, they were omitted from Table 1.

Jerome & Müller (2) reported that recovery of resistance in bean pods after heat treatment was governed by the temperature at which treated pods were stored before inoculation. To test the effect of temperature on recovery of resistance in heat-treated soybean plants, 30 Harosoy 63 plants were heat-treated at 44 C for

TABLE 1. Effect of temperature/time combinations on heat-induced susceptibility to *Phytophthora megasperma* var. *sojae* in Harosoy 63 soybean plants

Heat treatment		Time between heat-treatment and inocula-tion (hr)	% Plants	
			Dead	Infected
C	*min*			
44	15	0	55	70
44	15	24	0	0
44	30	0	85	90
44	30	24	0	0
44	60	0	100	100
44	60	24	0	0
45	15	0	45	75
45	15	24	0	0
45	30	0	60	70
45	30	24	20	30
47	15	0	70	75
47	15	24	30	75
47	30	0	100	100
47	30	24	20	70
50	¼	0	0	0
50	½	0	40	60
50	½	0	0	20
50	1	0	70	80
50	1	24	5	10
50	2	0	80	100
50	2	24	50	95
50	2	48	45	75
50	2	96	80	100
55	¼	0	0	0
55	1	0	100	100
55	1	24	0	0
55	2	0	100	100
55	2	24	100	100

30 min. Ten of these plants were stored at 28 C for 24 hr, 10 were stored at 10 C for 24 hr, and 10 were stored at 10 C for 48 hr. Each lot of plants was inoculated with *P. megasperma* var. *sojae* immediately after the storage period and incubated at 28 C. Ten Harosoy (susceptible) plants were inoculated as a check on pathogenicity of the inoculum.

The results 7 days after inoculation for the different temperatures and recovery periods were as follows: 28 C for 24 hr, none infected; 10 C for 24 hr, 90% infected; and 10 C for 48 hr, 90% infected. Obviously, low temperature suppressed the recovery of resistance in heat-treated soybean plants.

Effect of heat treatment beyond the treated area.—Tests were made to determine whether the effects of heat treatment extend beyond the tissues actually treated. Roots and hypocotyls of 40 Harosoy 63 plants were immersed in a water bath to a point 3 cm below the cotyledonary node. Four lots, 10 plants/lot, were inoculated at 3, 2, and 1 cm below the cotyledonary node, and at the node, respectively. All plants were incubated at 28 C.

Seven days after inoculation, all plants inoculated 3, 2, and 1 cm below the cotyledonary node were susceptible, but those inoculated at the node were resistant. Evidently, heat treatment did not affect the plants systemically.

As a further check, heat treatment was restricted to the upper 4 cm of the hypocotyl in two lots of Harosoy 63 plants. One lot was inoculated 2 cm below the cotyledonary node with *P. megasperma* var. *sojae;* the second lot was inoculated 7 cm below the node. In the first lot, all plants were dead within 3 days; the second lot showed no infection 7 days after inoculation, further demonstrating the localized effect of heat treatment. However, since the air temperature immediately above the water bath did not exceed 32 C, it is evident that the treatment induced a slight over-running effect in the hypocotyl tissues at least 2 cm beyond the treated portion.

DISCUSSION.—The effect of temperature duration appears to be of little importance at the 44 C level, but it changes critically between 45 and 47 C, especially with respect to the reversibility of induced susceptibility. The change at the 50 C level is even more critical, since 1 min induces reversible susceptibility, with the plant eventually regaining the ability to produce phytoalexin, whereas 2 min induces irreversible susceptibility, with the implication that the potential for producing phytoalexin has been lost.

Paxton & Chamberlain (3) found evidence of two types of resistance to *Phytophthora megasperma* var. *sojae* in soybeans: resistance in young (0-14 days old) plant tissues in which the production of phytoalexin plays an important role; and resistance in older (21-day-old) plant tissues characterized by woody stem tissues and decreased phytoalexin production. Since my investigations were obviously concerned with the first type, heat treatments and inoculations were completed before the plants were 14 days old. Otherwise, recovery of resistance would not reflect recovery of the ability to produce phytoalexin; rather, it might be confused with the morphological resistance of the older plant type, especially in plants 21 days old.

Of the two types of resistance, the one involving phytoalexin is the more important because it protects resistant seedlings at the stage of development when susceptible plants die in 24-48 hr after infection.

The extremely short lag period between inoculation and infection in the soybean-*Phytophthora* interaction makes it possible to approximate the time of recovery of resistance more precisely than would be possible with a slower-acting pathogen.

LITERATURE CITED

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