

Relationship of Age of Soybean Seedlings and Inoculum to Infection by *Pythium ultimum*

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ABSTRACT

The relationship of age of plant and inoculum to infection by *Pythium ultimum* was studied by inserting 4- to 20-day-old mycelium into the hypocotyl of soybean seedlings 8-14 days of age. Nearly 100%, 8-10 days of age, were killed with 4- to 8-day-old mycelium. The percentage killed progressively decreased with increased age of mycelium and/or increased age of plant. *Phytopathology* 61: 439-440.

Additional key words: *Glycine max*, root rot.

With the widespread use of *Phytophthora* root rot-resistant cultivars, other root rot problems have become more noticeable in soybeans, *Glycine max* (L.) Merr. Schmitthenner (4) studied the fungi associated with root necrosis of soybeans resistant to *Phytophthora megasperma* var. *sojae*. Most of the infected roots yielded *Pythium ultimum* Trow. Hildebrand & Koch (2) first reported *P. ultimum* on soybean in 1951. They found it affecting a number of cultivars in Essex and Kent counties in southwestern Ontario.

We inoculated most of the germplasm collection of the U.S. Regional Soybean Laboratory, amounting to 2,156 introductions, with *P. ultimum*. Several lines appeared to possess seedling resistance. One resistant plant introduction in maturity group II and one in maturity group III were crossed with highly susceptible commercial cultivars to study the inheritance of resistance. The F₁ plants were grown in the field, and the F₂ populations were inoculated in the greenhouse. Segregation results were inconclusive. Furthermore, repeatable results were not obtained with the susceptible and resistant parents from test to test. The age of the plants when inoculated (approx 10 days) and temperature (20-24 C) were kept fairly constant. The length of time the inoculum was grown before using was variable. A study was made to determine if the age of inoculum and/or the age of the plants when inoculated could be responsible for the discrepancies noted.

Breeders seed of the *Phytophthora* root rot-resistant cultivar Harosoy 63 was used. The seed was planted in nonsterilized soil covered with 1.5 cm of sand in 10-cm clay pots. Five dates of planting were made to obtain seedlings 8, 9, 10, 11, and 14 days of age.

Inoculum was grown at 22 C on oatmeal agar (OMA) prepared as described by Gooding & Lucas (1). My-

celial growth was rapid on this medium, and the mat was sufficiently tough in 4-5 days to use for inoculation. Mycelium of *P. ultimum* was transferred to OMA in 90-mm petri plates 4, 7, 8, 9, 10, 11, 14, 16, and 20 days before plants were to be inoculated.

Inoculum was prepared by scraping the agar medium from the underside of the mycelial mat and cutting the mat into pieces 2 × 3 mm. Plants were inoculated by inserting a piece of mycelium into a 1-cm longitudinal incision in the hypocotyl midway between the soil line and the cotyledons. The incision was covered with petroleum jelly to prevent desiccation.

Each treatment consisted of 20 plants; the test was repeated 3 times. In the first two tests, the plants were inoculated and maintained in the greenhouse at 24 C. Plants in the third test were placed in a controlled climate chamber at 15 C after inoculation.

Wilting and dying of plants was faster at 24 than at 15 C, but the final results from the three tests were essentially the same and are combined in Table 1. Nearly 100% of the plants 8-10 days of age were killed with inoculum grown 4-8 days. Inoculum grown for 9 days was effective on plants 8-9 days old but somewhat less effective on 10-day-old plants and much less effective on plants 11 and 14 days old. Inoculum grown for 10 days was not satisfactory on plants of any age. Plants 11 days old had a slightly lower percentage killed; whereas, 14-day-old plants had a much lower percentage killed with the most effective inoculum (4-8 days' growth). Plants more than 10 days old apparently are more difficult to infect with *P. ultimum*. We also have found this true with *Phytophthora megasperma* var. *sojae*, and Mellano et al. (3) reported that plants of *Antirrhinum majus* 25 days old developed a tolerance to *P. ultimum* that persisted for life.

We have maintained stock cultures of *P. ultimum* with periodic transfer for several years with no noticeable change in virulence. The length of time that inoculum can be grown before use appears to be short and critical for good infection. The results suggest that testing for resistance should be done with inoculum grown 4-8 days and plants 8-10 days old.

TABLE 1. Percentage of plants killed when mycelium of *Pythium ultimum* varying in age was inserted into the hypocotyl of soybean seedlings 8-14 days old

Age of inoculum (days)	% Killed when inoculated at indicated age ^a (days)				
	8	9	10	11	
4	100	100	100	71	19
7	100	98	96	87	17
8	100	97	98	85	6
9	100	99	78	51	5
10	58	49	48	40	18
11	40	43	38	35	6
14	32	28	33	30	6
16	24	15	24	18	5
20	8	7	15	3	0

^a Average of three tests with 20 plants each.

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