Interrelationship of Heterodera glycines and Phytophthora megasperma var. sojae in Soybeans

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ABSTRACT

Soybean (*Glycine max*) seedlings (2-, 5- and 10-days-old) of three cultivars varying in susceptibility to race 3 of the soybean cyst nematode (*Heterodera glycines*) and race 1 of *Phytophthora megasperma* var. *sojae* (*Pms*) were inoculated with each organism alone and in combination. Seedling disease was more severe in the *Pms*-susceptible cultivars Corsoy and Dyer when both organisms were present, than when *Pms* alone was present. *Pms*-resistant Harosoy-63 did not develop symptoms in the presence of both organisms. Mechanical wounding of seedlings did not appreciably influence resistance. *Pms*-infection significantly reduced the population of *H. glycines* on roots of Corsoy but did not break resistance of Dyer to the nematode. *Pms* appeared to be the dominant pathogen in the disease complex.

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The soybean cyst nematode, *Heterodera glycines* Ichinohe, causes yellowing and stunting of plants (1, 2, 17, 18, 19). *Phytophthora megasperma* Drechs. var. *sojae* Hild. (*Pms*) causes a pre- and postemergence damping-off of soybean seedlings, and a root and stem rot that causes plants at any age to wilt and die (5, 7, 9, 15). Both of these are important pathogens in the U.S. when either occurs alone (1, 17, 18). The potential damage to soybean [*Glycine max* (L.). Merr.] seedlings when both pathogens occur on the same plant has not been studied.

Wyllie and Taylor (20) showed that Harosoy soybeans inoculated with *Phytophthora sojae* Kaufmann & Gerdemann and *Meloidogyne hapla* Chitwood had a greater reduction in plant height, dry weight, and postemergence kill than plants inoculated with either pathogen alone. Ross (13) reported that Jackson soybeans developed severe wilt symptoms when grown in soil containing *H. glycines* and *Fusarium oxysporum* Schlecht., but not in soil containing either pathogen alone.

We report on the influence that *H. glycines* has on the development of soybean seedling disease caused by *P. megasperma* var. *sojae* (*Pms*).

MATERIALS AND METHODS.—The biotypes of the pathogens studied were race 1 of *Pms,* isolated from diseased, field-grown soybean plants in Champaign County, Illinois and race 3 of *H. glycines,* isolated from a field of soybean in Franklin County, Illinois. The soybean cultivars used were: Corsoy, susceptible to *Pms* and *H. glycines;* Dyer, susceptible to *Pms* and resistant to *H. glycines;* and Harosoy-63, resistant to *Pms* and susceptible to *H. glycines*. For each experiment, seeds that had been immersed in 0.5% sodium hypochlorite solution for 4 minutes were germinated for 2 days in sterile culture plates containing moist filter paper. One seedling, approximately 3 cm in length, was transplanted to each 10-cm diameter clay pot containing autoclaved sandy-loam soil.

Second-stage larvae of *H. glycines* were obtained from greenhouse-grown Clark-63 soybean. Soil in individual pots was infested by pouring 1 ml of an aqueous nematode suspension of approximately 500 larvae into a conical hole 2 cm deep. Seedlings were transplanted immediately into the same hole.

Frequent reisolations of *Pms* were made from greenhouse-grown soybean seedlings to maintain a high degree of virulence. Inoculum was produced on diluted lima bean agar (LBA) (2.3 g Difco LBA per liter distilled water) in sterile culture plates at 25 C for 10 days (9).

Soil in individual pots was infested with *Pms* at planting time, or 5-10 days later. In the first case, infestation of soil with *Pms* preceded inoculation with *H. glycines*. Inoculum and agar from a single culture plate was blended for 10 seconds in 20 ml of sterile distilled water in a food blender and mixed with 400 g of soil for each pot in a twin-shell dry blender. Pots infested after planting had a test tube (1 cm diameter) placed adjacent to each seedling at a depth of 1 cm so that infestation could proceed without mechanical damage to established roots. The test tube was removed at the time of infestation and a 4-mm agar disk of inoculum from the margin of a 10-day-old LBA culture was placed adjacent to the seedling hypocotyl, 1 cm below the soil line. The three soybean cultivars each were subjected to eight treatments with ten replications as follows: *H. glycines* alone at planting time; *Pms* alone at planting time, or 5 or 10 days after planting; *H. glycines* + *Pms* at planting time; *H. glycines* 5 or 10 days before *Pms*; and noninoculated control pots, which received 10 cc of sterile LBA to approximate that added with the inoculum.

The effect of mechanical wounding on the expression of *Pms* infection in the absence of *H. glycines* was also studied. Two-, 5-, or 10-day-old seedlings of the three soybean cultivars were either nonwounded or wounded on the hypocotyl 1 cm below the soil line by a single puncture with a flamed needle. A 4-mm agar disk of

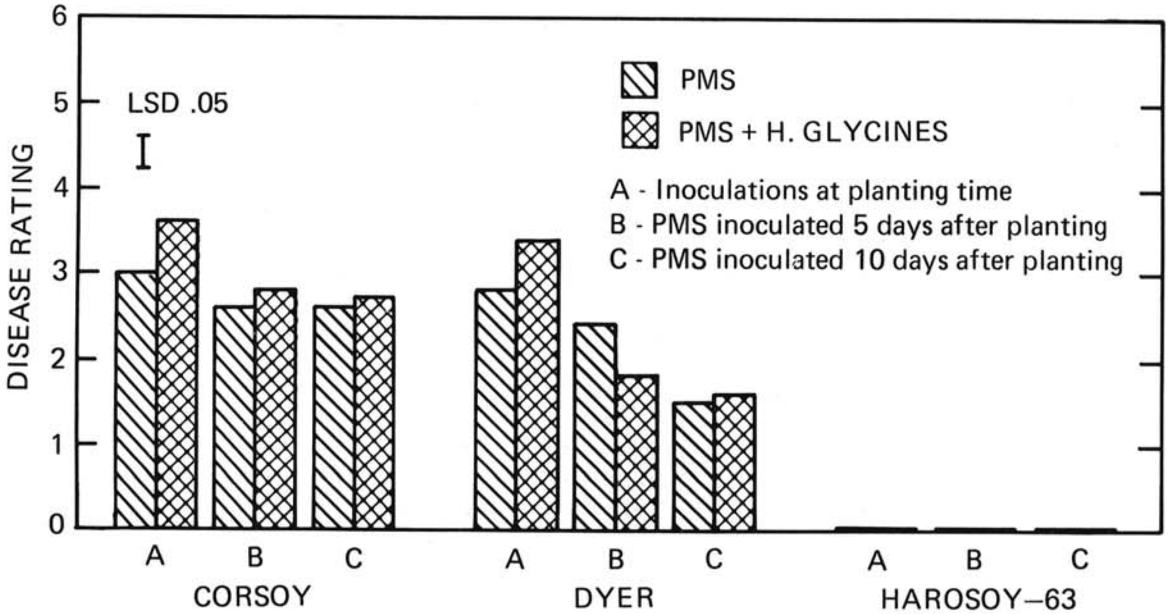

Fig. 1. Mean disease ratings of 10 plants from each of three soybean cultivars inoculated with either *Phytophthora megasperma* var. *sojae* (*Pms*) alone or in combination with *Heterodera glycines*. 1 = mild infection - small (20-30 mm) portion of tap root decayed; 2 = moderate infection - portions of both tap root and stem region decayed; 3 = severe infection - tap root, secondary roots and stem badly decayed; and 4 = pre- or post-emergence damping-off and death of the plant.

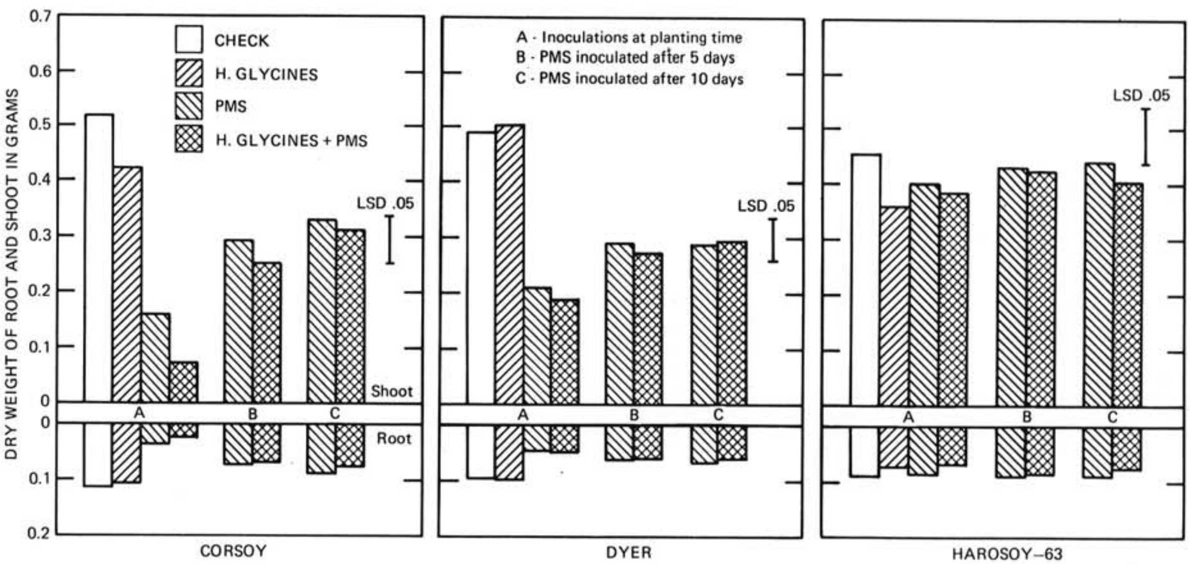

Fig. 2. Dry weights of roots and shoots from three soybean cultivars 30 days after inoculation with *Phytophthora megasperma* var. *sojae* (*Pms*) either alone, in combination with *Heterodera glycines*, or at 5 or 10 days after inoculation with *H. glycines*.

inoculum was placed adjacent to the seedling hypocotyl of all seedlings as described above. There were 20 seedlings for each treatment.

Pots in all experiments were placed in a transparent greenhouse growth chamber maintained at 25 C and illuminated during a 14-hour day with fluorescent lights. Daily observations were made for wilt, damping-off, and stem-rot symptoms. After 30 days roots of surviving plants were washed free of soil and rated as follows: 0 = no root infection; 1 = mild root infection - small portion (20-30 mm) of tap root decayed; 2 = moderate infection - portions of both tap root and basal stem region decayed; 3 = severe infection - tap root, secondary roots and stem badly decayed; and 4 = pre- and post-emergence damping-off with death of the seedlings. Counts were made of cysts and pre-cyst females on roots and those recovered from soil by decanting a soil-water suspension through a 246 μm (60-mesh) sieve. Roots and shoots were

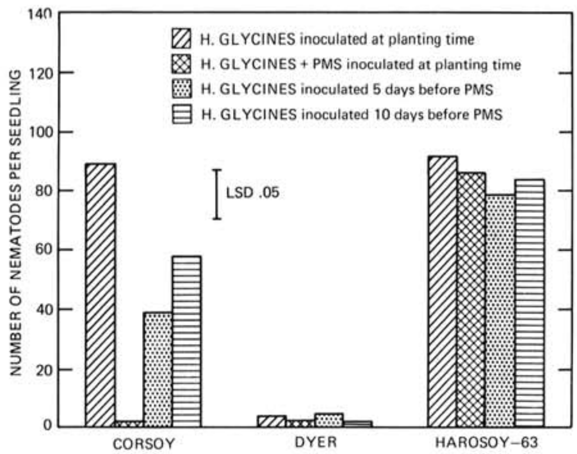

Fig. 3. Number of nematodes per soybean seedling in three cultivars inoculated with *Heterodera glycines*, alone or in combination with *Phytophthora megasperma* var. *sojae* (*Pms*) or at 5 or 10 days before inoculation with *Pms*.

dried separately for 4 days at 70 C, then weighed.

RESULTS AND DISCUSSION.—*P. megasperma* var. *sojae* penetrates roots of resistant and susceptible soybeans directly, and quickly establishes a pathogenic relationship (10, 11, 16). In our research, symptoms of *Pms* infection developed rapidly on Corsoy and Dyer, but no symptoms occurred on Harosoy-63 at any age after inoculation with either pathogen alone or in combination (Fig. 1). The disease rating of Corsoy and Dyer seedlings was generally higher for those inoculated with both pathogens than among seedlings inoculated with *Pms* alone. Disease ratings were higher on seedlings inoculated with both pathogens at the time of planting than on seedlings inoculated only with *Pms* 5 or 10 days after planting. Development of seedling disease or reduction in plant growth caused by *Pms* did not differ significantly ($P = 0.05$) between Corsoy and Dyer seedlings in the presence or absence of *H. glycines* (Fig. 1 & 2). Dyer seedlings inoculated 5 or 10 days after planting had a significantly lower disease rating than those inoculated at the time of planting, suggesting that resistance develops with age (Fig. 1). For both Corsoy and Dyer seedlings inoculated at the time of planting, 70% damped-off before the end of the experiment.

The reduction of shoot dry weights of Corsoy and Harosoy-63 seedlings inoculated with *H. glycines* alone approached significance ($P = 0.05$), but there was no reduction on Dyer seedlings (Fig. 2). Shoot dry weights of Corsoy and Dyer seedlings were reduced significantly when inoculated with either *Pms* alone or in combination with *H. glycines*. The differences lessened as seedling age at the time of inoculation increased. Shoot dry weights of seedlings did not differ significantly whether inoculated with *Pms* alone or with *Pms* plus *H. glycines*, except on Corsoy inoculated at planting time. Root dry weights of all cultivars inoculated with *Pms* alone or in combination with *H. glycines* generally were less than those of the controls, but the differences were not significant. Meyer and Sinclair (10) showed that *Pms* reduced the root systems of both fungus-resistant and susceptible soybean plants in field and greenhouse experiments of longer duration.

Infection by *Pms* did not favor the development of *H. glycines* on seedling roots (Fig. 3). Reduction in the number of nematodes on roots of Corsoy seedlings inoculated with *Pms* at all stages of plant development was highly significant. Most female nematodes that entered roots before infection by *Pms* failed to mature. Instead, they often turned brown prematurely and failed to produce eggs. Whether this decrease in nematode population was caused by reduction in the food base, or by unfavorable changes in the host physiology, is not known. *Pms* did not appear to break resistance of Dyer to the nematode, since there were no differences in nematode numbers among the treatments in this variety.

Mechanical wounding did not affect *Pms* infection of

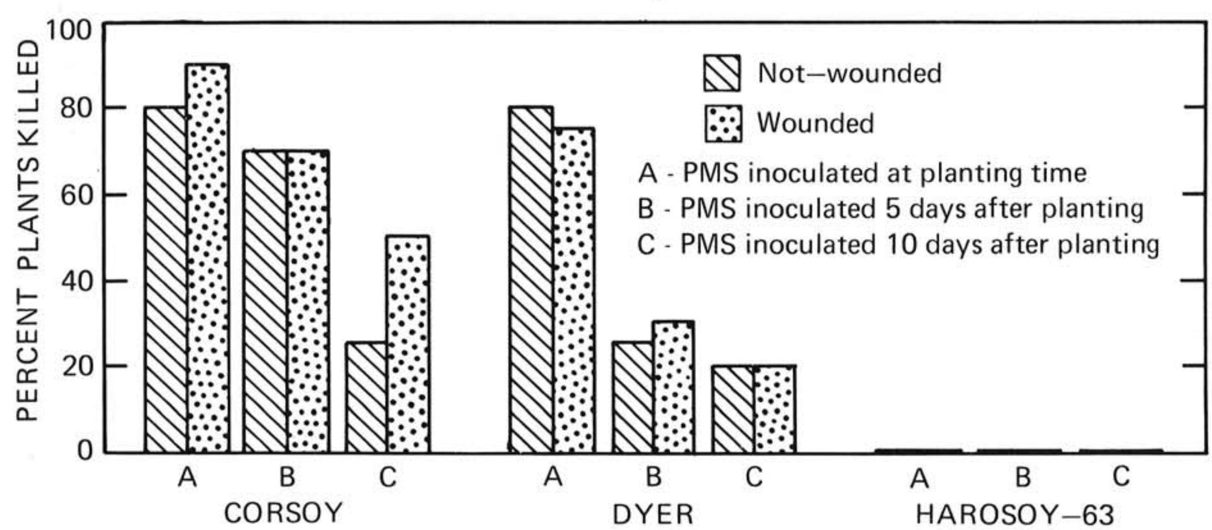

Fig. 4. Percent mortality of soybean seedlings in three cultivars after wound inoculation with *Phytophthora megasperma* var. *sojae* (*Pms*) at planting time or at 5 or 10 days after planting.

the three cultivars (Fig. 4). This finding agrees with that of Sasser et al. (14), who reported that mechanical wounding of tobacco plants did not increase the incidence of black shank caused by *Phytophthora parasitica* var. *nicotianae*. Jenkins and Coursen (6) reported that mechanical wounding of roots also did not influence the susceptibility of wilt-susceptible and resistant tomatoes to *Fusarium oxysporum* f. *lycopersici* and they suggested that factors other than nematode-induced wounds were involved in the nematode-wilt complex. Reynolds and Hanson (12), however, found that mechanical wounding and root-knot nematode infections served as debilitating factors for increased severity of *Rhizoctonia* damping-off of cotton seedlings. Haglund and King (4) suggested that a modification of host physiology may be involved in increased severity of pea root rot caused by *Aphanomyces euteiches* in the presence of *Tylenchorhynchus martini*. It has been shown that resistance of soybean to *Pms* depended upon phytoalexin production (3, 8, 11). Our observations indicate that phytoalexin production was not influenced by nematode infection.

Our results suggest that the increase in disease severity in treatments with both *Pms* and *H. glycines* was no more than additive. The dominant pathogen appeared to be *Pms,* which is considered to be the major contributing factor to losses from this disease complex on soybean. The nematode did not break the fungus resistance of Harosoy-63 and, conversely, the fungus did not alter the nematode resistance of Dyer. However, the fungus can severely inhibit population increase of *H. glycines* in cultivars susceptible to both pathogens.

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