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Population Development of *Meloidogyne incognita* on Soybean Defoliated by *Pseudoplusia includens*[1]

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Abstract: Greenhouse studies examined population densities of *Meloidogyne incognita* race 4 on soybean (*Glycine max* 'Davis') defoliated by larvae of soybean looper (*Pseudoplusia includens* (Walker)). Plants were defoliated over a 2-week period beginning 5 weeks after seedlings were transplanted. Four groups of plants were infested with nematodes (5,000 eggs/pot) at 2-week intervals to allow harvesting of plants at 0, 2, 4, and 6 weeks postdefoliation (WPD). Plants in each group were harvested 4 weeks after nematode infestation. Root and nodule weights of defoliated plants were suppressed at 0 WPD, but differences were not detectable at 2, 4, and 6 WPD. Population densities of *M. incognita* were similar on defoliated and control plants at 0 WPD but were greater on defoliated plants at 4 and 6 WPD. Percentage hatching of eggs produced on the latter plants also was higher. Effects of insect-induced defoliation on development of *M. incognita* remained detectable even after soybean plant growth apparently returned to normal.

Key words: Glycine max, insect defoliation, nematode, *Pseudoplusia includens,* root-knot nematode, soybean, soybean looper.

Soybean (*Glycine max* L. (Merrill)) in the southeastern United States is attacked by more than 19 species of insects and pathogens (16). *Meloidogyne* spp., among which *M. incognita* (Kofoid & White) Chitwood is the most common, are important members of this group throughout the region (19). During 1984–87, annual losses for soybean due to these and other nematodes averaged $73.1 million (13,14,15). Soybean looper (*Pseudoplusia includens* (Walker)) is an important migratory pest of soybean and is a member of a defoliator complex that caused losses of more than $37 million in 1984 (8).

Meloidogyne spp. usually attack soybean root systems throughout the growing season (19). Soybean usually is attacked by soybean looper during the host plant reproductive stages but can be damaged throughout the growing season by other insect defoliators, including velvetbean caterpillar (*Anticarsia gemmatalis* Hübner), green cloverworm (*Plathypena scabra* (F.)), and bean leaf beetle (*Cerotoma trifurcata* (Forster)) (20). The objective of this research was to evaluate population development of *M. incognita* in soybean as influenced by insect-induced defoliation.

Materials and Methods

Experiments were conducted in a greenhouse under supplemental fluorescent lighting (14 h light:10 h dark) at an intensity of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. Plants were grown in a sandy loam soil fumigated with a 67% methyl bromide, 33% chloropicrin mixture at a rate of 0.91 kg/1.42 m^3 soil. Before planting, soil received 0-24-24 (N-P-K) commercial fertilizer (0.76 g/kg soil) and aluminum sulfate (6 g/kg soil) according to standard nutrient and pH recommendations. Seeds of soybean 'Davis' were inoculated with commercial *Bradyrhizobium japonicum* (Kirchner) Buchanan before planting in flats. Eight days later, seedlings were transplanted individually to 3.2-liter plastic pots that contained 2.5 kg dry soil.

Meloidogyne incognita race 4 was cultured on tomato 'Rutgers' in a greenhouse. Inoculation with nematodes was accomplished by pipetting a 1-ml egg suspension (5,000 eggs) into a depression (2 cm wide × 4 cm deep) made in soil adjacent to the soybean

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stem. The depression then was filled with soil. Defoliation by insects was achieved by infesting individual plants with soybean looper larvae and allowing them to feed until approximately 45–50% of the leaf area was consumed (11,12).

In Experiment 1, treatments consisted of a single level of nematode infestation (5,000 eggs per pot), two levels of insect infestation (0 and 25 neonate larvae per pot), and four times of nematode infestation. These eight treatment combinations were replicated five times. Individual seedlings were transplanted into 40 pots on 3 June 1988. At the V5 (4) growth stage (35 days after transplanting), plants in 20 pots were infested with neonate soybean looper larvae, and the remaining 20 pots were not infested and served as controls. Insects were allowed to feed for 2 weeks. All plants then were sprayed with acephate (4.8 g a.i./l water) to kill the insect larvae. At this time, the number of insect larvae present on each plant was ca. 20% of the initial infestation level; the others succumbed to undefined mortality factors. This mortality was anticipated and accounted for in the initial infestation level (11).

Pots were divided into four groups of 10 pots each (five defoliated, five control). Plants in group 1 were infested with nematodes on 24 June (2 weeks before insect infestation) and harvested 4 weeks later on 22 July (0 weeks postdefoliation [0 WPD]). Thus, nematodes developed the first 2 weeks on plants before defoliation and the next 2 weeks on plants that were being defoliated. Plants in group 2 were infested with nematodes on 8 July (the day of insect infestation) and harvested on 5 August (2 WPD). Nematodes in this group developed the first 2 weeks on plants that were being defoliated and the next 2 weeks on plants that were recovering from defoliation. Plants in group 3 were infested with nematodes on 22 July and harvested on 19 August (4 WPD). These nematodes developed on plants that were recovering from defoliation for 4 weeks. Plants in group 4 were infested with nematodes on 5 August and harvested on 2 September (6 WPD). Nematodes developed on plants that were recovering from defoliation for 6 weeks.

Plants in each group were harvested 4 weeks after nematode infestation by severing stems at the cotyledonary node. Root systems were shaken gently to free them from soil. Fresh weights of roots and shoots were determined immediately after harvest. Galling of root systems was rated using a 0–5 severity scale (2). Leaves were removed from plants, and leaf area per plant was determined using an area meter (Li-Cor model LI-3100). Soil samples (250 cm^3) were collected from each pot. Second-stage juveniles were extracted from soil using a modified sugar-flotation technique (9). Root subsamples (1 g) removed at random from each root system were placed in NaOCl (0.525%) for 10 minutes to remove nematode eggs from the gelatinous matrix. Eggs were incubated in deionized water in Baermann funnels under ambient laboratory light and temperature conditions. The percentage hatch for these eggs was determined by counting egg hatch over 14 days.

Experiment 2 was a duplicate of Experiment 1, except for minor changes. At the V4 growth stage (29 days after transplanting), plants in one-half of the pots were infested with 10 fourth-instar soybean looper larvae. Insects were maintained on the plants for 2 weeks, by which time defoliation was completed and larvae had pupated. This infestation procedure differed slightly from that in Experiment 1, in which neonate larvae were used. However, most (ca. 93%) leaf consumption is done by the fourth and later instars (10). Therefore nearly all defoliation in Experiments 1 and 2 was accomplished by late-instar larvae over a period of about 6 days. Pots were divided into four groups of 10 pots each, and plants in each group were infested with nematodes and harvested after 4 weeks as described for Experiment 1. Number of nodules on roots and total nodule fresh weight per plant were recorded in addition to parameters measured in Experiment 1.

Both experiments were established ac-

cording to a completely random design with factorial arrangement of treatments. Data were analyzed using GLM of SAS (18) to test for main treatment effects. Experiments 1 and 2 were considered as blocks for statistical analysis. Factors beyond our control caused premature mortality of a few plants in Experiment 1; therefore, treatments were compared using least squares means (18). Significant differences between treatments are expressed at $P \leq 0.05$.

Results and Discussion

At 0 WPD, leaf areas of plants infested with insects were reduced 45% relative to control plants that were not infested (Table 1). Leaf areas on defoliated plants still were smaller at 4 WPD but recovered to levels of controls at 6 WPD (Table 1). Root and nodule fresh weights were less ($P < 0.05$) in defoliated plants at 0 WPD but not later; however, shoot fresh weights and number of nodules per plant were not affected (Table 1). In a similar study, Layton and Boethel (12) showed clearly that recovery of nitrogen-fixing ability in Davis soybean following insect-induced defoliation was due to recovery of total nodule weight and nodule specific activity (μmol $C_2H_4 \cdot g^{-1} \cdot h^{-1}$). In the present study, total nodule weight recovered to a level similar to that in controls by 2 WPD. Although we did not measure nodule specific activity, the 45% defoliation in our study was below the 66% defoliation necessary for reduction of nodule specific activity in this cultivar (11). Therefore, the rapid recovery from insect-induced defoliation in our study is due to low initial leaf area loss coupled with quick recovery of nodule weights.

Numbers of root galls and second-stage juveniles were greater on defoliated plants at 4 and 6 WPD, and egg production on roots of these plants was enhanced at 6 WPD (Table 2). These enhancements of *M. incognita* population parameters, especially at 4 and 6 WPD, indicate that effects of the insect-induced defoliation in soybean remain long after plant growth has returned to normal. In a related study (17), stepwise increases in insect-induced defoliation of soybean 'Bragg' resulted in concomitant increases in populations of *Heterodera glycines* in roots and soil. The similar results from both studies with different nematode species and host cultivars may indicate a general pattern for nematode population development on roots of soybean plants being subjected to insect-induced defoliation. In addition, nematodes on soybean roots also can influence development of foliage-feeding insects. Al-

Table 1. Growth of soybean 'Davis' not defoliated (control) or defoliated by larvae of soybean looper (*Pseudoplusia includens*).

Weeks postdefoliation†	Treatment	Leaf area (cm^2)	Fresh weight (g)			Nodule no.‡
			Root	Shoot	Nodule‡	
0	Control	476	13.0	5.0	1.54	40.4
	Defoliated	261	7.6	3.8	0.86	30.8
	$P > F$	0.0002	0.0010	0.1919	0.0333	0.2561
2	Control	929	31.0	8.7	4.26	60.8
	Defoliated	716	30.5	7.0	3.22	42.6
	$P > F$	0.0688	0.9321	0.0805	0.1252	0.1264
4	Control	1416	26.9	12.9	5.06	113.4
	Defoliated	1069	23.6	10.7	4.92	109.8
	$P > F$	0.0321	0.2355	0.0727	0.8254	0.8446
6	Control	1673	34.3	19.1	9.14	133.4
	Defoliated	1359	29.8	16.3	7.68	96.4
	$P > F$	0.1216	0.2590	0.2302	0.1481	0.0936

Values presented are least squares means.
† 0, 2, 4, and 6 weeks after 2-week period of insect infestation.
‡ Data obtained from Experiment 2 only.

TABLE 2. Population parameters of *Meloidogyne incognita* race 4 after development on soybean 'Davis' for 4 weeks as affected by soybean looper (*Pseudoplusia includens*) defoliation.

Weeks postdefoliation†	Treatment	Root gall index‡	Eggs/g root tissue	Juveniles/250 cm^3 soil	Egg hatch %
0	Control	3.8	5131	69	45.6
	Defoliated	3.8	5351	91	78.2
	$P > F$	0.8574	0.8488	0.1130	0.0234
2	Control	2.6	1577	103	47.4
	Defoliated	3.1	4331	164	48.3
	$P > F$	0.2304	0.0838	0.3882	0.8583
4	Control	1.9	1508	66	27.5
	Defoliated	3.7	3060	120	64.7
	$P > F$	0.0003	0.0673	0.0383	0.0048
6	Control	1.3	564	199	25.2
	Defoliated	3.5	5342	486	53.8
	$P > F$	0.0001	0.0001	0.0194	0.0120

Values presented are least squares means.
† 0, 2, 4, and 6 weeks after 2-week period of insect infestation.
‡ Root system galled: 0 = 0%; 1 = 10%; 2 = 20%; 3 = 55%; 4 = 80%; 5 = 100%.

ston et al. (1) reported greater numbers of corn earworm larvae on soybean in response to open canopies caused by high populations of *H. glycines*. Very high populations of nematodes, however, caused plants to be stunted and chlorotic, thus reducing their suitability as food for insect larvae (1).

Nematode egg hatch was stimulated on defoliated plants at 0, 4, and 6 WPD (Table 2). This result suggests a relationship between insect-induced defoliation and dormancy in eggs of *M. incognita*. Increased production of dormant eggs by *Meloidogyne* spp. was reported as the growing season progressed and under conditions of environmental stress (3,6,7,21). Results from the present study, however, were in contrast to these reports but in agreement with previous studies (1,17). Orthogonal polynomial contrasts (5) failed to show a relationship between egg hatch and plant age (data not presented). Egg hatch was increased in response to defoliation stress. Factors responsible for these effects have not been identified. The contrasts among research results (1,3,6,7,17,21) may be due, in part, to differences in host plant species or biotic and abiotic stresses.

Under field conditions, *Meloidogyne* spp. attack host root systems throughout the growing season. Defoliating insects also can be a problem but may be particularly damaging during soybean reproductive stages. Therefore, the need is evident for full-season studies to thoroughly examine the relationships between these pests and their damage potential.

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