Effects of Growth Regulators and Nematodes on Cylindrocladium Black Root Rot of Soybean

B. A. FORTNUM, Assistant Professor, and S. A. LEWIS, Associate Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29631

ABSTRACT

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Meloidogyne incognita, when inoculated on Davis soybean, increased the damage of Cylindrocladium black root rot (CBR) to soybean. Application of ethephon and chlormequat chloride to *M. incognita*-infected soybean increased the severity of CBR, and severity of CBR in *M. incognita*-inoculated soybean was related to the time of fungus inoculation. The influence of the *M. incognita* life cycle on CBR development is discussed.

Additional key words: kinetin, naphthalene acetic acid

The pathogen *Cylindrocladium crotalariae* (Loos) Bell & Sobers causes a root rot of soybean (*Glycine max* (L.) Merr.), and disease development is influenced by the southern root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood. Soybean infected with *M. incognita* interacted with *C. crotalariae* and more disease subsequently developed than in nematode-free plants; greatest root necrosis was observed when *M. incognita* was applied 14 days before inoculation with *C. crotalariae* (M. Hedrick, *unpublished*).

Plant growth regulators have been implicated in the response of plant tissue to root-knot nematode invasion (6,7,11). Application of exogenous kinetin (6) or a combination of kinetin and auxin (6,11) stimulated gall development and giant cell formation in plants resistant to *Meloidogyne* spp. Orion and Minz (7) noted an increase in fresh weight of *M. javanica* galls caused by the addition of ethephon, an ethylene generating compound. Neither giant cell formation nor the number of females per gall was affected when ethephon was added to the plant. The increased gall size resulted from parenchyma tissue proliferation (7). Akitt et al (1) showed that tomato roots infected with *M. incognita* contain significantly less ethylene per gram than healthy roots. Inhibitors of gibberellin synthesis, such as chlormequat chloride, can retard nematode development, which may be an indirect effect of the growth regulator on plant growth (14,15). Hormones responsible for molting in insects have shown plant growth regulator activity, and conversely, gibberellic acid stimulates the developmental rate of locusts (3). The molting of *Meloidogyne* spp. is coincident with the hypertrophy and hyperplasia associated with *Meloidogyne* galls. Comparisons between hormonal regulated insect molting and nematode development have been indirect. There is evidence, however, of a general sensitivity of these organisms to certain plant growth regulators.

Modification of growth hormone levels during root-knot nematode establishment and their influence on nematode development and root-rotting fungi remain unexplored. The purpose of this study was to determine 1) the effect of *M. incognita* on disease caused by *C. crotalariae* on soybean, 2) the effect of growth regulators on the interaction of *M. incognita* and *C. crotalariae* on soybean, and 3) the influence of growth regulators on nematode development and the pathogenicity of *C. crotalariae*.

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MATERIALS AND METHODS

Culture of plants and preparation of inoculum. Soybean seeds (cultivar Davis) were inoculated with *Rhizobium japonicum* (Kirchn.) Buch. and germinated in vermiculite. Five-day-old seedlings were transplanted into 15-cm plastic pots containing 1,500 cm^3 of a 50% sand-Varina sandy loam mixture. Similarly grown seedlings were used in field microplots containing Varina sandy loam. Soils for greenhouse and microplots were fumigated with methyl bromide (0.1 kg/0.2 m^3). The Race 3 (12) *M. incognita* population was isolated from soil obtained at the Clemson University Edisto Experiment Station (Blackville, SC) and cultured on tomato seedlings (*Lycopersicon esculentum* Mill. 'Marion'). Nematode eggs from 35-day-old tomato roots extracted in 0.5% sodium hypochlorite and washed in tap water served as inoculum (5). A root suspension filtrate obtained from uninfected plants was added to control plants. All *M. incognita* inoculations were made by adding a suspension of eggs around the soybean roots at transplanting.

C. crotalariae was cultured in a 4% malt broth (Difco) for 6 wk at 22 C. A 25-ml broth culture of *C. crotalariae* was comminuted with a blender in 100 ml of tap water and microsclerotia were collected on a 35-μm sieve. The microsclerotia were resuspended in tap water and added as a 25-ml soil drench to appropriate containers in the greenhouse experiment. Inoculum was prepared for microplots by seeding autoclaved (sterile) wheat seeds with a 7-mm mycelial disk of *C. crotalariae* and incubating them for 14–28 days at 22 C. Field microplots were infested with *C. crotalariae* by placing 2 g of fungus-colonized wheat seed into two holes 4–6 cm deep next to the base of each soybean plant.

Effects of growth regulators on disease. Treatments applied to soybean seedlings in the greenhouse study consisted of *M. incognita* (5,000 eggs per 15-cm pot) and *C. crotalariae* (5,000 microsclerotia per 15-cm pot), singly or in combination with the separate growth regulator treatments: chlormequat chloride (American Cyanamid Co.), ethephon (GAF Corp.), or naphthalene acetic acid (NAA) sodium salt, pH 7.5 (Sigma Chemical Co.), plus kinetin (Fisher Scientific Co.) dissolved in hot water. Chlormequat chloride and NAA plus kinetin were applied at concentrations of 10 and 100 ppm per growth regulator. Ethephon was applied at concentrations of 2,000 and 4,000 ppm. Growth regulators were applied by wick-feeding 5 ml of the solution to the bases of two soybean stems (9). Control treatments were wick-fed sterile distilled water. Growth regulators and *C. crotalariae* were applied 14 days after transplanting. Nematodes were added at transplanting. Soil temperature was maintained in the greenhouse in constant temperature baths at 27 ± 1 C, and fresh and dry stems and root weights were recorded after 75 days. Root necrosis was rated on a 0–5 scale: 0 = no necrosis, white root system; 1 = tan discoloration;

2 = necrotic lesions on root surface; 3 = root severely necrotic but no reduction in size; 4 = severe necrosis with few feeder roots; and 5 = root system reduced in size and coal black. A randomized complete block design with four replicates per treatment was used and the test was repeated once.

Influence of *M. incognita* and seedling age on CBR disease. Soybean seedlings (five plants per 60-cm spherical field microplot) were inoculated with 40,000 *M. incognita* eggs per microplot at transplanting and with *C. crotalariae* 0, 4, 16, and 32 days after transplanting. A randomized complete block design with four replicates per treatment, including single inoculations of *M. incognita, C. crotalariae*, and uninoculated controls, was used for the test. Fresh and dry weights of roots and stems, galling indices, and root necrosis were determined 75 days after transplanting.

RESULTS

Effects of growth regulators on disease. Ethephon (2,000 ppm) significantly increased the root necrosis of Davis soybean inoculated with *M. incognita* and *C. crotalariae* (Table 1). Ethephon (4,000 ppm) increased root necrosis caused by *C. crotalariae* in nematode and nematode-free treatments. Root growth, however, was severely retarded by ethephon alone. Plants treated with ethephon and *M. incognita* had significantly more root necrosis than those with ethephon alone, even in the absence of *C. crotalariae*. A combination of ethephon plus *M. incognita* and *C. crotalariae* further enhanced root degeneration, and the application of ethephon caused an increase in gall size.

Chlormequat chloride (10 ppm) significantly increased root necrosis and suppressed the shoot weight of soybean inoculated with *M. incognita* and *C. crotalariae* (Table 1). Treatments at higher concentrations of chlormequat chloride exhibited similar trends. Addition of NAA plus kinetin increased root necrosis of *C. crotalariae*-inoculated plants, but this was not significant.

Influence of *M. incognita* and seedling age on CBR disease. *M. incognita* significantly ($P = 0.05$) increased susceptibility of soybean to *C. crotalariae* (Table 2). Root necrosis and reduction in plant growth was greatest when soybeans were inoculated simultaneously with *M. incognita* and *C. crotalariae* (Table 2).

DISCUSSION

Ethylene (ethephon) and antigibberellin (chlormequat chloride) application during an *M. incognita* infection increased damage by *C. crotalariae*. This is evident because root necrosis was more severe on *M. incognita*-infected soybeans to which ethephon and chlormequat were applied. Changes in plant growth hormones during *Meloidogyne* spp. infection have been reported with implications of involvement in the nematode life cycle (6,11). Growth regulator application at nontoxic concentrations exerted little influence on *C. crotalariae* development in the absence of *M. incognita*. We conclude that ethylene and gibberellin levels influence nematode-fungus interactions but that the observed interaction was dependent on nematode-induced changes other than a simple hormone imbalance.

Growth, differentiation, and cell metabolism are controlled by a balance of growth hormones. Meloidogyne root galls are rich in organic metabolites and have an altered tissue composition (8). Van Gundy et al (13) reported increased root leakage of tomato infected by *M. incognita* and a corresponding increase in susceptibility to *Rhizoctonia solani*. Growth hormones, however, can alter membrane permeability (2) and may play a role in increasing leakage of altered root tissue.

Involvement of NAA and kinetin in

Table 1. Dry shoot weight and root necrosis index of Davis soybean after inoculation with *Cylindrocladium crotalariae, Meloidogyne incognita*, and treatment with growth regulators

Treatment[w,x,y]	Concentration of growth regulators (ppm)	Root necrosis[z] index	Shoot weight in grams
	Without *M. incognita*		
Control	···	0.8 fg	8.5 ab
Ethylene (ethephon)	2,000	1.0 efg	2.6 efg
Ethylene (ethephon) + *C. crotalariae*	2,000	2.4 cde	2.2 fg
Antigibberellin (chlormequat)	10	1.2 efg	8.2 ab
Antigibberellin (chlormequat) +*C. crotalariae*	10	1.8 cdefg	5.3 cd
NAA and kinetin	10	1.0 efg	8.4 ab
NAA and kinetin + *C. crotalariae*	10	2.0 cdef	8.7 a
C. crotalariae	···	1.4 defg	7.4 abc
	With *M. incognita*		
Control	···	0.4 g	6.1 abc
Ethylene (ethephon)	2,000	2.8 bcd	2.4 efg
Ethylene (ethephon) + *C. crotalariae*	2,000	5.0 a	0.3 g
Antigibberellin (chlormequat)	10	1.0 efg	5.9 abcd
Antigibberellin (chlormequat) + *C. crotalariae*	10	3.8 ab	2.1 fg
NAA and kinetin	10	1.2 efg	6.4 abc
NAA and kinetin + *C. crotalariae*	10	3.2 bc	3.4 def
C. crotalariae	···	2.2 cdef	4.9 cde

[w] Data analyzed by Duncan's multiple range test. All values not followed by the same letter within a column are significantly different ($P = 0.05$).
[x] Growth regulators in 5-ml bottles were wick-fed to the base of the plant. *Cylindrocladium crotalariae* was added at a rate of 5,000 microsclerotia per 15-cm pot. *Meloidogyne incognita* (5,000 eggs per 15-cm pot) was applied at transplanting.
[y] Soil temperature was maintained at 27 C during the 72-day experiment.
[z] Necrosis rated on a 0–5 scale with 5 = maximum necrosis.

Table 2. Fresh shoot weight and root necrosis index of Davis soybean grown in microplots and inoculated with *Cylindrocladium crotalariae* 0, 4, 16, and 32 days following inoculation with *Meloidogyne incognita*

C. crotalariae inoculation	Root necrosis index[x,y]	Shoot weight in grams
	Without *M. incognita*	
At planting	0.3 de	27.6 b
4 days	0.6 bcde	29.7 ab
16 days	0.4 cde	46.0 a
32 days	0.1 e	26.5 b
Control	0.6 bcde	35.0 ab
	With *M. incognita*[z]	
At planting	1.9 a	20.1 b
4 days	1.0 bcde	21.0 b
16 days	1.4 ab	26.2 b
32 days	1.1 bc	26.8 b
Control	0.2 e	35.4 ab

[x] Necrosis rated on a 0–5 scale with 5 = maximum necrosis.
[y] Data analyzed by Duncan's multiple range test. All values not followed by the same letter within a column are significantly different ($P = 0.05$).
[z] *Meloidogyne incognita* was added as an egg suspension at transplanting (40,000 eggs/container). *Cylindrocladium crotalariae* was added as infected wheat seed (20 g/container).

nematode development and plant resistance to root-knot nematodes has been reported (6,11,15). NAA and kinetin increase establishment and maturation of *M. incognita* in susceptible and resistant tomato seedlings. The resistance mechanism altered by NAA and kinetin, however, appears to be unrelated to the increased susceptibility of soybean to *C. crotalariae* during an *M. incognita* infection because application of NAA and kinetin failed to increase Cylindrocladium black root rot significantly in any treatment.

Several mechanisms (10) have been suggested to explain the increased susceptibility of root-knot infected plants to soil fungi. Wounding induced by *M. incognita* may favor Cylindrocladium root rot development; inoculation of *M. incognita* and *C. crotalariae* in field microplots showed the greatest damage when both organisms were applied together. M. Hedrick (*unpublished*) noted both an increase in the level of plant mortality with simultaneous inoculation of *M. incognita* and *C. crotalariae* and an increased level of root necrosis when *C. crotalariae* was applied 14 days after *M. incognita* inoculation. Powell (10), however, observed enhanced Fusarium wilt of tobacco in plants pretreated with *Meloidogyne* spp. and suggested that a more elaborate mechanism than simple wounding may also be occurring. Diomonde and Beute (4) similarly noted increased Cylindrocladium black root rot of peanut with *M. hapla* and suggested that other mechanisms in addition to wounding may affect fungal disease development.

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