IN VITRO SCREENING OF SOYBEAN LINES TO *RADOPHOLUS SIMILIS* AND *R. CITROPHILUS*

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Accepted:
28.VII.1989
Aceptado:

ABSTRACT

Huettel, R. N. 1989. In vitro screening of soybean lines to *Radopholus similis* and *R. citrophilus*. Nematrópica 19:143–149.

One population each of *Radopholus similis* and *R. citrophilus* from Florida were screened using root explant cultures to determine their reproduction potential on the soybean (*Glycine max*) cultivars, Peking, Bragg, Centennial, Pickett, Lee, Kent, Essex, Bedford, Delmar, Hill, Williams, Dyer, Hood, and PI 90763. After a 5-week incubation at 25 C, counts of adults/juveniles and eggs were determined in roots that had been removed from the agar and stained in acid-fuschin. The black-seeded lines, 'Peking' and PI 90763 known to be resistant to other nematode species were highly susceptible to *R. citrophilus* and moderately susceptible to *R. similis*. Counts from the other 12 cultivars were, in general, lower for *R. similis* than *R. citrophilus*. Furthermore, by plotting of root weight to the number of eggs and mixed life stages recovered from the roots, the degree of tolerance of any of the tested soybean cultivars to the two nematode species could be determined.

Key words: burrowing nematode, *Glycine max*, host status, *Radopholus citrophilus*, *R. similis*, resistance, root-explant culture.

RESUMEN

Huettel, R. N. 1989. Determinación in vitro de la respuesta de lineas de soya a *Radopholus similis* y *R. citrophilus*. Nematrópica 19:143–149.

Utilizando cultivos de trozos de raíz, se determinó la reproducción potencial de una población de *Radopholus similis* y una población de *R. citrophilus* originarias de Florida en los cultivares de soya (*Glycine max*), Peking, Bragg, Centennial, Pickett, Lee, Kent, Essex, Bedford, Delmar, Hill, Williams, Dyer, Hood, y PI 90763. Luego de 5 semanas de incubación a 25 C, se efectuaron recuentos de adultos/larvas y número de huevos en las raíces extraídas del agar y teñidas con fuccina ácida. Las lineas de semillas negras 'Peking' y PI 90763, resistentes a otros especies de nematodos, resultaron ser muy susceptibles a *R. citrophilus* y moderadamente susceptibles a *R. similis*. Recuentos efectuados en otros 12 cultivares fueron, en general, menores para *R. similis* que para *R. citrophilus*. Además, se determinó el grado de tolerancia de todos los cultivares de soya ensayados a las dos especies de nematodos graficando número de huevos y recuento de otros estados de desarrollo recuperados de las raíces en relación al peso de raíz.

Palabras claves: cultivos con trozos de raíz, *Glycine max*, hospedantes, nematodo minador, *Radopholus citrophilus*, *R. similis*, resistencia.

INTRODUCTION

In vitro screening for resistance of cultivars to plant-parasitic nematodes was first demonstrated for root-knot nematodes, *Meloidogyne* spp. in the 1960's (2–4). More recently, root-explant cultures have been used for evaluating resistance of soybean (*Glycine max* (L.) Merr.) to soybean cyst nematodes (*Heterodera glycines* Ichinohe) (13), of grape (*Vitis vinifera* L.) to *Pratylenchus vulnus* Allen & Jensen (18) and of peach (*Prunus persica* (L.) Batsch) to *Meloidogyne incognita* (Kofoid & White) Chitwood (9). To date, the use of in vitro culturing techniques has been well demonstrated as a rapid and reliable tool for determining reproductive capabilities of plant-parasitic nematodes on known hosts, new hosts, resistant cultivars or novelly-produced plant cultivars from tissue culture (9,10,17).

In this study, in vitro screening techniques were used to determine resistance of soybean cultivars to the citrus and banana burrowing nematodes, *Radopholus citrophilus* Huettel, Dickson & Kaplan and *R. similis* (Cobb) Thorne. These species have been reared successfully using several in vitro culturing methods (7,8,11), thus making in vitro host resistance studies possible. The objectives of this research were two-fold. The first objective was to develop an in vitro screening method for the two *Radopholus* species to determine their reproductive potential on 14 soybean lines. The second goal was to determine if the degree of host suitability could be established by measuring reproductive capabilities of the two species on each soybean lines.

MATERIALS AND METHODS

Nematode cultures: Radopholus similis and *R. citrophilus* were obtained from established in vitro cultures as described previously by Huettel et al. (6). These populations were maintained on corn (*Zea mays* L.) root explants on Gamborg's B-5 medium (Grand Island Biological Co, Grand Island, New York, U.S.A.) prior to inoculating on soybean lines (8).

Soybean root-explant cultures: The following soybean lines were used: cvs. Hill, Lee, Centennial, Hood, Williams, Delmar, Kent, Bragg, Pickett, Bedford, Essex, Dyer, Peking, and PI 90763. Seeds from these lines were surfaced sterilized and germinated on 1.5% water agar. Two days after germination, 2–3 cm root tips, two per plate, were transferred to Gamborg's B-5 medium (8) in 50 × 150-cm-diam petri plates. All root-explants of these lines grow well under culture conditions (Huettel, unpubl.).

Nematode inoculation and counts: Both species were extracted from explants that were ca. 60 days old. The roots were removed from the agar, cut into small pieces, and aerated in water for 24 hours. The nematodes were collected in centrifuge tubes and surface sterilized (8).

Aliquots containing 50–75 nematodes (mixed life stages) were inoculated aseptically onto 4-day-old soybean root explants. Three petri plates per cultivar were used and the experiment repeated four times with each trial serving as a replication.

After 5 weeks, roots were removed from the petri plates by heating them over boiling water for 5 minutes to soften the agar. Fresh root weights were recorded and the roots were stained immediately in acid fuschin (1). Nematodes were extracted from the roots (17) and the number of nematodes (adults and juveniles) and the number of eggs were recorded.

Data analysis: Nematode counts were log transformed before analysis. All data were subjected to an analysis of variance and means were separated using least significant difference ($P \leq 0.05$).

RESULTS

The results of this study indicated that both *Radopholus* species were able to reproduce on all fourteen soybeans lines tested. The number of eggs/g of root and the number of nematodes/g of root extracted after 5 weeks from roots infected with *R. citrophilus* and *R. similis* are listed in Tables 1 and 2, respectively. The black-seeded cultivar, Peking supported the highest number of eggs and nematodes of all lines for both nematode species. The other black-seeded line, PI 90763, also had a high number of both eggs and nematodes for *R. citrophilus* and high count of nematodes for *R. similis*. Lower counts of both eggs and nematodes were observed for both species on cultivars that generally

Table 1. Number of eggs and nematodes (adults/juveniles) recovered 5 weeks postinfection from soybean root explant cultures inoculated with 50–75 adults/juveniles of *Radopholus citrophilus*.

Cultivar	Nematodes/g of root	Eggs/g of root
Peking	3 336	5 215
PI 90763	2 138	817
Essex	1 970	1 811
Dyer	1 063	358
Bedford	1 059	572
Bragg	811	626
Delmar	701	612
Williams	701	655
Hood	698	222
Lee	582	237
Pickett	500	352
Centennial	368	210
Kent	137	101
Hill	100	60
LSD (0.05)	1 654	1 954

Data are means of four replications, three petri plates each.

Table 2. Number of eggs and nematodes (adults/juveniles) recovered 5 weeks postinfection from soybean root-explant cultures after inoculation with 50–75 adults/juveniles of *Radopholus similis*.

Cultivar	Nematodes/g of root	Eggs/g of root
Peking	1 973	3 136
Bragg	1 462	2 355
PI 90763	1 447	2 343
Pickett	305	416
Centennial	360	389
Lee	197	300
Bedford	172	290
Kent	171	194
Essex	190	193
Hood	167	173
Delmar	167	166
Williasms	119	163
Dyer	101	133
Hill	115	128
LSD (0.05)	1 630	947

Data are means of four replications, three petri plates each.

are considered to be very susceptible to other plant-parasitic nematodes (Tables 1 and 2).

Radopholus citrophilus had, in general, an overall higher reproduction rate on the soybean cultivars tested when compared to *R. similis*. The highest counts on 'Peking' were 3 336 and 3 136 for eggs/g of root and 5 215 and 1 973 nematodes/g of root for *R. citrophilus* and *R. similis*, respectively. The lowest number of eggs/g root and nematodes/g of root was recovered from 'Hill' for *R. citrophilus* and 'Dyer' and 'Hill' for *R. similis*. The means were 60 and 100, and 101 and 128, respectively.

The response of each cultivar was evaluated using scatterplots of the number of eggs and nematodes to fresh root weights (Fig. 1,2). Cultivars that appeared to have a very sensitive reaction were indicated by low fresh root weight and high nematode counts. This is illustrated by reproduction of *R. citrophilus* on 'Peking' (Fig. 1). In other cases, such as 'Kent' with *R. citrophilus* (Fig. 1) and with *R. similis* (Fig. 2), the number of nematodes and eggs recovered were moderate in number, but the root weight was high. This indicated a less sensitive reaction to the nematode infection. These scatterplots show that in general, most lines are less sensitive to *R. similis* (Fig. 2) (indicated by greater root weights and lower nematode counts) than to *R. citrophilus* (Fig. 1).

DISCUSSION

Soybeans have been reported to be a host of both *R. similis* and *R. citrophilus* (14,15). In this study, in vitro screening was demonstrated as a good technique for rapid determination of reproduction capabilities

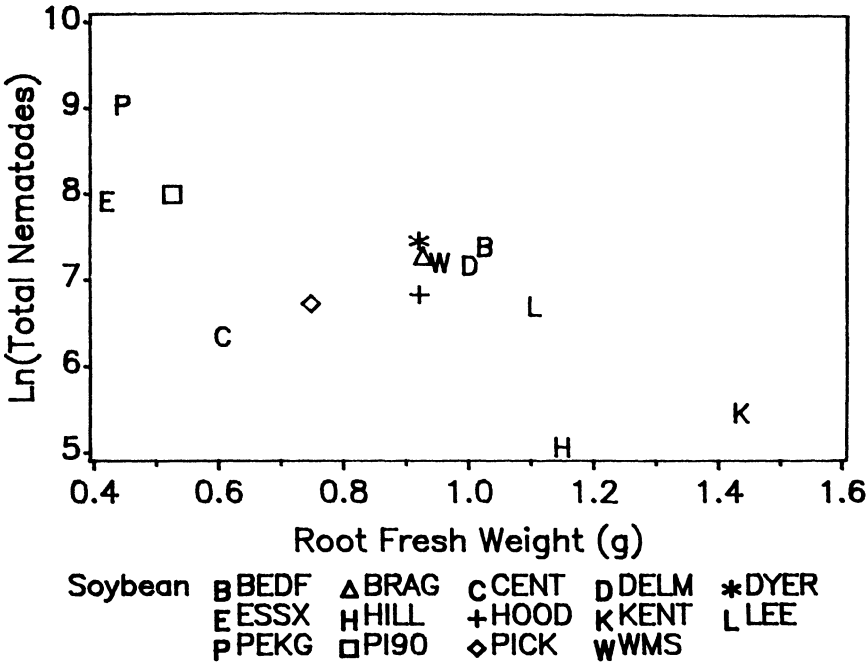

Fig. 1. Scatterplots of eggs and nematodes (mixed life stages) per g of fresh root weight for *Radopholus citrophilus* (mean of four replications, three plates each).

of *Radopholus* spp. on soybean lines. Scatterplots may be useful in identifying lines that are tolerant to *R. similis* and *R. citrophilus*. The use of scatterplots may be valuable also for evaluating other migratory endoparasitic nematodes, such as the lesion nematode, *Pratylenchus* spp., when using in vitro screening techniques.

It is interesting to note that the black seeded cultivar, Peking is a resistant parent line used to confer nematode resistance in soybean to *Heterodera glycines* and *Rotylenchulus reniformis* Linford & Oliveira (18). Some of the other lines tested are resistant to several species of root-knot nematode as well as some races of the soybean cyst nematode (5,18). Some of these lines were very susceptible to both species of *Radophlus*. This could present a severe problem if resistant cultivars to both root-knot and cyst nematodes were introduced into an area which has been infested previously with either *Radopholus* species.

In the development of new soybean lines for new areas, such as subtropical and tropical climates, nematode resistance should be considered in breeding programs (12). Furthermore, all important nematode species associated with other crops in the area should be considered as potential problems for new soybean cultivars.

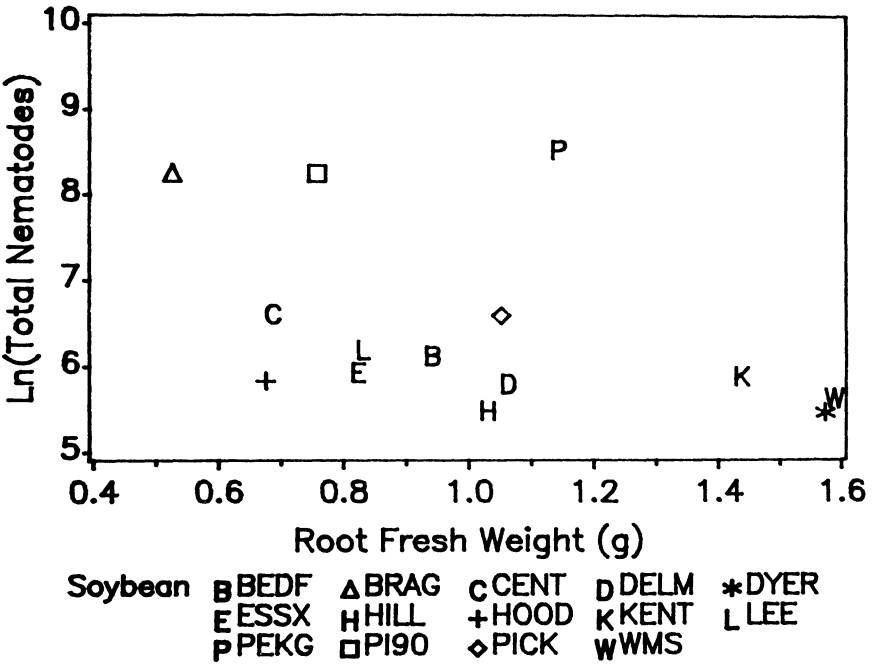

Fig. 2. Scatterplots of eggs and nematodes (mixed life stages) per g of fresh root weight for *Radopholus similis* (mean of four replications, three plates each).

LITERATURE CITED

1. BRIDGE, J. S., L. PAGE, and S. M. JORDAN. 1981. An improved method for staining nematodes in roots. P. 173 *in* Rothamsted Report for 1981. Rothamsted Experimental Station, Harpenden, Herts., England.
2. DROPKIN, V. H., and W. R. BOONE. 1966. Analysis of host-parasite relationships of root-knot nematodes by single larva inoculations of excised tomato roots. Nematologica 12:225–236.
3. DROPKIN, V. H., and R. E. WEBB. 1967. Resistance of axenic tomato seedlings to *Meloidogyne incognita acrita* and *M. hapla*. Phytopathology 57:584–587.
4. DROPKIN, V. H., D. W. DAVIS, and R. E. WEBB. 1967. Resistance of tomato to *Meloidogyne incognita acrita* and to *M. hapla* (root-knot nematodes) as determined by a new technique. Proceedings of the American Society of Horticultural Science 90:316–323.
5. FASSULIOTIS, G. 1982. Plant resistance to root-knot nematodes. Pp. 33–49 *in* R. D. Riggs, ed. Nematology in the Southern Region of the United States. Southern Cooperative Series Bulletin 276. Arkansas Agricultural Experiment Station. Fayetteville, Arkansas, U.S.A.
6. HUETTEL, R. N., D. W. DICKSON, and D. T. KAPLAN. 1984. *Radopholus citrophilus* n. sp., a sibling species of *Radopholus similis*. Proceedings of the Helminthological Society of Washington 51:32–35.
7. HUETTEL, R. N. 1985. Carrot disc culture. Pp. 154–155 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison, eds. Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experimental Station. Amherst, Massachusetts, U.S.A.
8. HUETTEL, R. N., and R. V. REBOIS. 1985. Culturing plant parasitic nematodes using root explants. Pp. 155–158 *in* B. M. Zuckerman, W. F. Mai, and M. B. Harrison,

eds. Plant Nematology Laboratory Manual. University of Massachusetts Agricultural Experimental Station. Amherst, Massachusetts, U.S.A.
9. HUETTEL, R. N., and F. A. HAMMERSCHLAG. 1986. Influence of cytokinin on in vitro screening of peaches for resistance to nematodes. Plant Disease 70:1141–1144.
10. HUETTEL, R. N., and F. A. HAMMERSCHLAG. 1988. Response of in vitro propagated peaches in culture, greenhouse, and microplots to *Meloidogyne incognita*. Phytopathology.
11. INSERRA, R., and J. H. O'BANNON. 1975. Rearing migratory endoparasitic nematodes in citrus callus and roots produced from citrus leaves. Journal of Nematology 7:261–263.
12. KIHL, R. A., L. A. DE AMEIDA, and A. DALL'AGNOL. 1985. Strategies for cultivar development in temperate climates Pp. 301–304 *in* R. Shibles, ed. World Soybean Research Conference III. Western Press: London.
13. LAURITIS, J. A., R. V. REBOIS, and L. S. GRANEY. 1982. Screening soybean for resistance to *Heterodera glycines* Ichinohe using monoxenic cultures. Journal of Nematology 14:593–594.
14. MILNE, D. L., and D. P. KEETCH. 1976. Some observations on the host plant relationships of *Radopholus similis* in Natal. Nematrópica 6:13–17.
15. O'BANNON, J. H. 1977. Worldwide dissemination of *Radopholus similis* and its importance in crop production. Journal of Nematology 9:16–25.
16. PALYS, J. M., and C. P. MEREDITH. 1984. Dual cultures of grape (*Vitis* sp.) and the lesion nematode (*Pratylenchus vulnus*) for studies of nematode tolerance. Plant Disease 68:1058–1060.
17. REBOIS, R. V., and R. N. HUETTEL. 1986. Population dynamics, root penetration, and feeding behavior of *Pratylenchus agilis* in monoxenic root cultures of corn, tomato, and soybean. Journal of Nematology 18:392–397.
18. RIGGS, R. D. 1982. Cyst nematodes in the southern U.S.A. Pp. 78–95 *in* R. D. Riggs, ed. Nematology in the Southern Region of the United States. Southern Cooperative Series Bulletin 276. Arkansas Agricultural Experiment Station. Fayetteville, Arkansas, U.S.A

Received for publication:
4.III.1989
Recibido para publicar: