EVALUATION OF *HOPLOLAIMUS COLUMBUS* SHER REPRODUCTION ON SELECTED SOYBEAN CULTIVARS USING GREENHOUSE AND EXCISED ROOT CULTURAL TECHNIQUES

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ABSTRACT

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Host suitability of six soybean cultivars to *Hoplolaimus columbus* was evaluated in monoxenic culture and greenhouse experiments. Differences in nematode population development were detected among cultivars. 'Bryan,' was less suitable as a host than 'Braxton,' and 'Centennial.' Suitability of these cultivars for reproduction of *H. columbus* did not relate to their maturity groups or tolerance rating, a result consistent with previous findings.

Key words: Columbia lance nematode, *Glycine max*, *Hoplolaimus columbus*, reproductive factor, resistance, soybean, tolerance.

RESUMEN

Supramana, S., S. A. Lewis, J. D. Mueller, B. A. Fortnum y R. E. Ballard. 2001. Evaluación de la reproducción de *Hoplolaimus columbus* Sher en variedades seleccionadas de soya mediante técnicas aplicadas en experimentos de invernaderos y cultivo de raíces. Nematrópica 31:267-271.

Se evaluó la aptitud de seis cultivares de soya como hospederas de *Hoplolaimus columbus* en cultivos monoxenicos y experimentos de invernaderos. Se detectó diferencias en el desarrollo de las poblaciones de nematodo entre variedades. 'Bryan' fue menos apropiada como hospedera que 'Braxton,' y 'Centennial.' La aptitud de de estos cultivares para la reproducción de *H. columbus* no estuvo relacionada con la madurez de los grupos o grado de tolerancia, lo cual concuerda con previos estudios.

Palbras claves: Factor reproductivo, *Glycine max*, *Hoplolaimus columbus*, nematodo lanceta de Columbia, resistencia, soya, tolerancia.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the primary hosts of the Columbia lance nematode (*Hoplolaimus columbus* Sher). In severely infested fields, plants are stunted, chlorotic, and have fewer pods, resulting in poor yields (Appel and Lewis, 1984; Fassuliotis *et al.*, 1968; Lewis and Smith, 1976; Mueller and Sanders, 1987; Noe, 1993). The application of nematicides is usually uneconomical, but management of *H. columbus* on soybean can be effectively and economically achieved with host resistance and/or tolerance.

Resistance to Columbia lance nematode has not been reported in soybean; however, several cultivars are tolerant to *H. columbus* in the field (Mueller *et al.*, 1988; Garner, 1990; Arslan, 1998). Cultivars reported to be tolerant include 'Dillon' (Maturity Group VI), 'Coker 6847,' 'Hagood,' and 'Stonewall' in Maturity Group VII, and 'Kirby,' 'Maxcy,' and NK's 'S83-30' in Maturity Group VIII (Drye *et al.*, 1997).

Nematode reproduction rates on soybean cultivars in the greenhouse and in monoxenic cultures may be a useful parameter in assessing host status for resistance screening. Reduced nematode reproduction could be responsible for prior reports of tolerance in the field. The success in culturing this nematode species on excised roots (Supramana and Lewis, 1998, 1999b) and in the greenhouse (Supramana and Lewis, 1999a) is essential for developing a greenhouse screening assay for resistance/tolerance. Our objective was to evaluate the host suitability of soybean cultivars to *H. columbus* using greenhouse and monoxenic culture techniques rather than field screening.

MATERIALS AND METHODS

Reproduction rates of *H. columbus* on six soybean cultivars, plus one negative control peanut cultivar and one positive control soybean (Hutcheson) were assessed in the greenhouse and on excised roots. Six cultivars from different maturity groups that were susceptible and/or tolerant to *H. columbus* were chosen. Bryan (Maturity Group VI), Braxton (VII), and Perrin (VIII) are considered intolerant, whereas Centennial (VI), Hagood (VII), and Coker 368 (VIII) are considered tolerant in *H. columbus*-infested fields (Mueller *et al.*, 1988; Garner, 1990; Arslan, 1998). 'Hutcheson' soybean (V) was also included in the experiment because this cultivar was an excellent host of *H. columbus* in previous studies (Supramana and Lewis, 1998, 1999a,b). In addition, a non-host crop, 'Georgia Green' peanut, was included in the greenhouse experiments.

Reproduction on Excised Root: Experiments were conducted: 1) December 1998 to March 1999 and 2) June to September 1999. Soybean seeds of each cultivar were surface sterilized by soaking in 95% ethanol and then transferred to 1.3% sodium hypochlorite, each for 10 minutes. The seeds were germinated on 1% water agar. A single root tip, 30 mm in length, was transferred to solidified Gamborg's B5 medium, pH 5.9 (Gamborg *et al.*, 1976) in 9-cm diameter petri plates containing 1.3% agar. Ten to 20 nematodes cultured on Hutcheson soybean roots were inoculated onto each root tip 7 days later. After double sealing with parafilm strips, the plates were inverted and placed in a 30°C incubator (Nyczepir and Lewis, 1976). The experiment was terminated 90 days after inoculation. The roots were removed from the petri plates and placed in a mist chamber (30 seconds mist every 2 minutes) for 7 days to extract nematodes from the roots. Nematodes that had migrated from the roots were collected and counted. The reproductive factor (Rf) for *H. columbus* was calculated by dividing the final population by the initial population. Experimental units were arranged in a completely randomized design with six replications of each of six soybean cultivars and one other soybean cultivar plus peanut as controls.

Reproduction in the Greenhouse: Two consecutive greenhouse experiments were conducted in spring and summer, 1999. Treatments were arranged in a 2 × 8 factorial with five replications. There were 80 experimental units (pots); 40 were inoculated and the other 40 were not. Hutcheson soybean and a non-host crop (Georgia Green peanut) were included in the experiment as positive and negative controls, respectively.

Before planting, seeds were treated with carboxin fungicidal seed treatment (Vitavax-3F). The experiments were conducted using 1-liter plastic pots filled with pasteurized gravel and river sand (1:3, v/v). Gravel, with particle diameter approximately 10-mm, was placed on the bottom of each pot, followed by double layers of plastic window screen and river sand on top. Pots were held in stainless steel boxes (28 × 20 × 10 cm, one box held two pots) by filling the box with river sand. Insulated metal boxes containing pots were placed in temperature controlled water baths adjusted to 30°C. Four seeds were planted directly into each pot. After emergence, plants were thinned to two per pot. Juveniles and adults of *H. columbus*, cultured on excised soybean roots, were pipetted into three 3-cm-deep holes around soybean roots 10 days after planting. The average initial populations of 218 and 277 nematodes per pot during the first and second experiments, respectively. The plants received 16 hours of supplemental light from a 400-W light bulb (Sylvania 400 W) placed at 1 m above the water bath. The experiment was terminated 60 days after inoculation. Mist chamber (as described above) and centrifugal-flotation (Jenkins, 1964) techniques were used to extract *H. columbus* from roots and sand, respectively. These numbers were combined to generate total nematode numbers. Fresh and dry weights of soybean shoots and roots were also recorded. Data were analyzed using analysis of variance (ANOVA), with Least Significant Difference (LSD) test and 5% alpha to separate the differences among the means.

RESULTS

Reproduction on Excised Root: The soybean cultivars varied in their suitability as hosts to *H. columbus* under monoxenic conditions (Table 1). Bryan, Coker 368, and Perrin were generally less suitable for nematode reproduction than Braxton and Centennial using monoxenic culture evaluation. Average reproductive factors (Rf) of 127.17 and 46.05 were obtained on Hutcheson (data not shown), the positive control, in the first and second experiments, respectively. This indicates that *H. columbus* reproduced well on excised soybean roots in both experiments. Reproduction was greater in the first experiment than in the second.

Table 1. Reproduction of *Hoplolaimus columbus* on excised roots of soybean 90 days after inoculation.

Soybean cultivar	Tolerance level[y]	Maturity group	*H. columbus* reproductive factor (Rf)[z] Exp. I	Exp. II
Bryan	-	VI	41.28 b	13.19 b
Centennial	+	VI	94.64 a	60.86 a
Braxton	-	VII	94.86 a	46.21 a
Hagood	+	VII	176.09 a	13.18 b
Perrin	-	VIII	40.28 b	23.82 ab
Coker 368	+	VIII	34.71 b	10.45 b

[y]Tolerant (+) or intolerant (-) to *H. columbus* in previous field experiments. Data are means of six replications. Means within a column followed by a common letter are not different (LSD = 0.05).
[z]Rf = number of nematodes at harvest divided by inoculum density.

Reproduction in the Greenhouse: Final populations of *H. columbus* varied significantly (LSD = 0.05) among cultivars (Table 2) and were generally greater in the second experiment than in the first. Reproduction on Perrin and Centennial was significantly greater than on Braxton and Bryan. In addition, an average Rf of 14.57 and 0.11 occurred on Hutcheson soybean and Georgia Green peanut, respectively, (data not shown in Table 2), indicating that *H. columbus* retained its host specificity.

Effect on Soybean Growth: Hoplolaimus columbus did not significantly reduce soybean growth after 60 days under greenhouse conditions in either experiment (data not shown). The overall mean shoot dry weights of inoculated plants were greater in both experiments, although not significantly different. However, *H. columbus* significantly reduced overall mean root dry weight in the second experiment where an approximate three-fold higher number of nematodes was recovered upon termination of the study.

DISCUSSION

Suitability of the soybean cultivars as hosts for *H. columbus* did not relate to their maturity groups or previous tolerance ratings. These results are consistent with previous findings (Arslan, 1998). However, soybean cultivars varied in their suitability as hosts for *H. columbus* in the greenhouse and on excised roots. Consistently lower nematode recovery occurred on Bryan, and higher reproduction generally occurred on Centennial. The excised root culture technique was more consistent in the results obtained over the two experiments than was the greenhouse experiment and is therefore preferable in that respect.

The adoption of excised root culture of *Hoplolaimus columbus* for assessing tolerance is problematical, since tolerance is typically assessed in the field. We have demonstrated the potential usefulness of *H. columbus* grown in tissue culture for determining host and cultivar suitability. If greenhouse/growth chamber environmental conditions can be designed to permit tolerance assessments, monoxenic culturing of *H. columbus* could be useful. Modifications might be needed to use excised root cultures in screening for true resistance, if it exists, since the optimal temperature for nematode growth of 30°C (Supramana and Lewis, 1998, 1999b) is also the temperature

Table 2. Reproduction of *Hoplolaimus columbus* on soybean in the greenhouse 60 days after inoculation.

Soybean cultivar	Tolerance level[y]	Maturity group	*H. columbus* recovered - Rf[z] Exp. I	Exp. II
Bryan	-	VI	954 a-4.4	3 104 b-11.2
Centennial	+	VI	1 538 a-7.1	5 034 a-18.2
Braxton	-	VII	1 490 a-6.8	3 004 b-10.8
Hagood	+	VII	1 678 a-7.7	3 832 ab-13.8
Perrin	-	VIII	1 486 a-6.8	5 634 a-20.3
Coker 368	+	VIII	1 754 a-8.1	4 670 ab-16.9

[y]Tolerant (+) or intolerant (-) to *H. columbus* in previous field experiments. Data are means of five replications. Means within a column followed by a common letter are not different (LSD = 0.05).
[z]Rf = number of nematodes at harvest divided by inoculum density.

at which a significant number of nematode resistance genes in some plants become sensitive to high temperature (Young, 1998). Monoxenic culture of *H. columbus* can provide pure populations that can be used in precise cultivar screening techniques. If tolerance could be linked to a plant growth characteristic, such as ability to produce extra roots (Trudgill and Cotes, 1983) or an association between tolerance for seed yield and tolerance for plant height (Boerma and Hussey, 1984), a protocol for greenhouse screening for tolerance could be developed. Until then, monoxenic culture can be useful for determining host status, or it can provide pure cultures of *H. columbus* for other screening procedures.

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