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Host Status of *Crotalaria juncea, Sesamum indicum, Dolichos lablab,* and *Elymus glaucus* to *Meloidogyne javanica*[1]

M. Araya and E. P. Caswell-Chen[2]

Abstract: Reproduction of *Meloidogyne javanica* on *Crotalaria juncea* PI 207657 and cv. Tropic Sun, *Sesamum indicum, Dolichos lablab,* and *Elymus glaucus* was assessed using a root-gall index, a reproductive index obtained by dividing the final population of juveniles (J2) in soil by the initial J2 population (Pf/Pi), and the number of J2 per gram of root recovered from roots by mist chamber extraction. *Lycopersicon esculentum* (cv. UC 204 C) was included as a susceptible host. The root-gall index and soil reproductive index were poor indicators of the host status of our test plants as compared with mist chamber extraction of J2 from roots. *Lycopersicon esculentum* had a mean root-gall index of 7.8. Some plants of *S. indicum* and *E. glaucus* had a few galls and other plants had none, with mean root-gall indices of 1.6 and 0.8, respectively. No galls were observed in *C. juncea* and *D. lablab. Lycopersicon esculentum* had the highest mean soil Pf/Pi value (mean = 1.93), while in *C. juncea* and some replicates of *S. indicum* no soil J2 were found. Even though some replicates had no galls, all replicates supported nematode reproduction. The mean numbers of J2 per gram root after 5 days of mist extraction were 447.7, 223.3, 165.5, 96.9, 42.3, and 41.9 for *D. lablab, L. esculentum, E. glaucus, S. indicum,* and *C. juncea* PI 207657 and cv. Tropic Sun, respectively. Accurate assessment of nematode resistance was influenced by sampling time and the nematode extraction technique used. Individual plants of both *C. juncea* and *S. indicum* supported nematode reproduction to some extent; however, both *C. juncea* and *S. indicum* have potential as cover crops to reduce *M. javanica* numbers.

Key words: Crotalaria, Dolichos, Elymus, host status, *Meloidogyne javanica,* nematode, nematode reproduction, Pf/Pi, reproductive index, resistance evaluation, root-gall index, *Sesamum.*

Meloidogyne javanica (Treub, 1885; Chitwood, 1949) is among the most economically destructive plant-parasitic nematodes, with a wide host range (6,7), and geographical distribution (10). *Meloidogyne javanica* is longer lived, is more adapted to environmental change, and competes more effectively than *M. incognita* (9). In California agriculture, *M. javanica* is an important limiting factor in crop growth (12).

Nematode control tactics include nematicides, cultural practices, resistant cultivars, and crop rotations. Because sustainable agricultural systems are ecologically based and beneficent of natural resources (4), future success in integrated nematode management will depend on combinations of control tactics that reduce nematode numbers without disrupting the agroecosystem. Specific crop and target nematode combinations will require unique arrays of control strategies (8,13–15).

The use of cover crops, which can be rotated between annual and biennial crops or interplanted among perennial crops, has been considered for nematode management. Plants with potential for control of root-knot nematodes include *Crotalaria* species (16), *Sesamum indicum* L. (13–15, 18), and *Dolichos lablab* L. (19). However, little information is available about their effects on *M. javanica,* and the mode of action by which they reduce *Meloidogyne* spp. numbers is unclear. Reduced root penetration of *C. juncea, S. indicum,* and *D. lablab* by *M. javanica* has been reported (1), but no data have been published on reproduction that help elucidate the mode of action of these plants in reducing nematode numbers. The objectives of this research were to assess the host status of *C. juncea, D. lablab, S. indicum,* and *Elymus glaucus* Buckl. to a California population of *M. javanica* using *Lycopersicon esculentum* Mill. as the susceptible control, and to determine how well a root-gall index, a re-

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[2] Graduate Research Assistant and Assistant Professor, respectively, Department of Nematology, University of California, Davis CA 95616. Address correspondence to second author.

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productive index (Pf/Pi) based on second-stage juveniles (J2) extracted from soil, and mist chamber extraction of J2 from roots characterized the reproductive potential of *M. javanica* on *C. juncea, D. lablab, S. indicum,* and *E. glaucus.*

Materials and Methods

Two entries of *Crotalaria juncea* (PI 207657, an accession from USDA Southern Regional Plant Introduction Station, Griffin, GA, and cv. Tropic Sun, [16], *S. indicum, D. lablab* cv. Highworth, *E. glaucus* cv. Berkeley, and *L. esculentum* cv. UC 204 C (Sunseeds Genetics Inc., Hollister, CA) were used in the experiment.

All seeds, except *E. glaucus,* were germinated on moist Whatman's No. 4 filter paper (9.0-cm-d) in a petri dish for 4 days at 30 C. Two germinated seeds were transplanted individually into each of five black plastic 53-liter pots filled with 79.5 kg sterilized, washed mortar sand. *Elymus glaucus* seeds (50 per pot) were germinated directly in the 53-liter pots. Before use, the sand was sterilized at 118 C under 1.05 kg/cm^2 pressure for 2 hours. All pots were kept in a lath house in a completely randomized design with six treatments and five replicates.

Plants were irrigated as needed with filtered water and fertilized twice a week with commercial Miracle-Gro fertilizer (Stern's Miracle-Gro Products, Inc., Port Washington, NY). When plants were 45 days old, they were inoculated with *M. javanica* juveniles (J2) obtained from a hydroponic culture (11), achieving an initial population density (Pi) of 1.3 J2/g sand in the pots. A total of ca. 106,000 J2 were added to the sand surface of each pot through three inoculations at 2-day intervals.

Ninety days after the last inoculation, plants were uprooted and roots were rinsed free of sand. A root-gall index (5) was estimated using a subjective index from 0 to 8, where 0 indicated roots were gall-free and 8 meant that roots had extremely heavy galling (ca. 100% galling) and poor root development. Second-stage juveniles were extracted from 250 g soil using a Baermann funnel for 3 days (2), and from 15.0-g root samples using a mist chamber for 24 days (2). Roots of *L. esculentum* were in the mist chamber for only 5 days because J2 extraction was negligible after 5 days. Nematodes from the Baermann funnels were rinsed into a dish and the J2 counted under the dissecting microscope (1–7×). A soil reproductive index of the final J2 density divided by the initial J2 density (Pf/Pi) was calculated. Data were log transformed and subjected to mean separation using the Waller–Duncan *k*-ratio *t* test (17).

Nematodes from the roots in the mist chamber were collected 3 days after the samples were set up and thereafter every 2 days, using a 20-μm-pore sieve. Juveniles were rinsed into a dish and counted using a dissecting microscope (1–7×). The cumulative nematode numbers per gram of root for each replicate at each collection time were recorded (Pf). The data lacked normality and homogeneity of variance, and were log transformed and subjected to mean separation using the Waller–Duncan *k*-ratio *t* test (17). The means of nontransformed data are presented.

Results

Lycopersicon esculentum had heavily galled roots (mean index = 7.8), with limited root development, whereas *C. juncea* PI 207657, cv. Tropic Sun, and *D. lablab* had no visible galls (Table 1). One replicate plant of *S. indicum* had many galls (index = 8.0) with limited root growth, resulting in a mean root-gall index of 1.6 for *S. indicum* (Table 1). Roots of two *E. glaucus* replicates had slight galling (index = 2.0), which resulted in a mean gall index of 0.8 (Table 1). With the exception of the *L. esculentum* and one galled replicate of *S. indicum,* roots of all other plants appeared healthy, with abundant feeder roots. On the basis of the root-gall index alone, many replicates within crops appeared resistant to *M. javanica.*

The soil reproductive index (Pf/Pi) differed among plants ($F = 4.71$, $P = 0.0039$). *Lycopersicon esculentum* had a sig-

TABLE 1. Root-gall index (0–8; where 0 = no galls and 8 = ca. 100% galling) for *Crotalaria juncea, Dolichos lablab, Elymus glaucus, Sesamum indicum,* and *Lycopersicon esculentum* 90 days after inoculation with an initial *Meloidogyne javanica* density of 1.3 J2/g sand (106,000 J2/53-liter pot).

Plant	Mean gall index	Standard error
Lycopersicon esculentum	7.8	0.2
Dolichos lablab	0.0	—
Elymus glaucus	0.8	0.5
Sesamum indicum	1.6	1.6
Crotalaria juncea PI 207657	0.0	—
Crotalaria juncea cv. Tropic Sun	0.0	—

Data are means of five replicates.

nificantly higher Pf/Pi than did the other plants (Fig. 1). The lowest mean Pf/Pi values were observed in *C. juncea* (cv. Tropic Sun = 0.01 and PI 207657 = 0.04), but neither *C. juncea* entry was significantly different from *D. lablab, E. glaucus,* and *S. indicum* (Fig. 1).

Even though many plants lacked galls, nematode reproduction was observed in all replicates (Fig. 2). *Dolichos lablab* had greater nematode numbers per gram of root (Pf) at all collection times than did any of the other plants, including *L. esculentum* for the first 5 days. Generally, the cumulative nematode population (Pf) increased with the number of days in the mist chamber. In plants with low nematode numbers, such as *C. juncea* and *S. indicum,* cumulative nematode numbers from roots in the mist chamber reached a maximum before 24 days.

Significant differences in the cumulative J2 numbers per gram of root were observed among plant species at each collection time (Table 2). *Dolichos lablab, L. esculentum,* and *E. glaucus* supported greater J2 numbers than did *S. indicum* and both *C. juncea* entries at 3, 5, or 7 days. After 9 days, plants were grouped slightly differently, and *S. indicum* was not significantly different from *D. lablab* and *E. glaucus,* as all appeared to be good hosts for *M. javanica* (Table 2). The average recovery across all plants was ca. 50% more at 7 days than at 5 days. After 7 days, the difference in cumulative nematode numbers between consecutive collection times decreased, but differences were still ca. 25% at 13 days. The average cumulative J2 numbers across

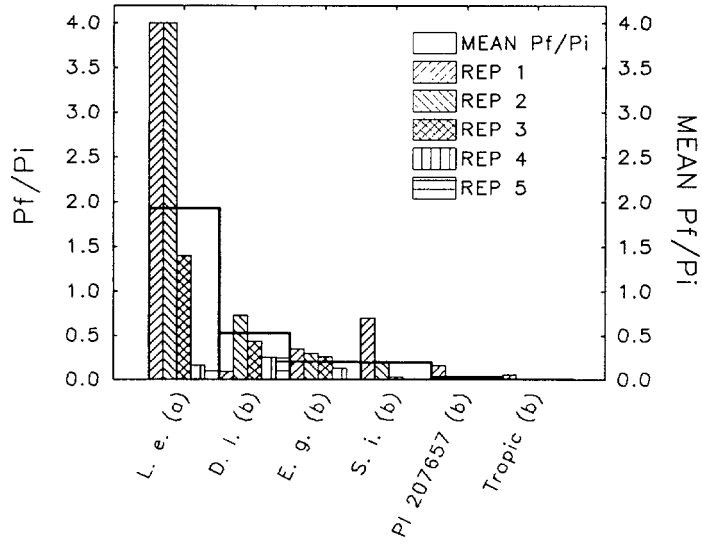

FIG. 1. Reproductive index calculated as the final second-stage juvenile (J2) density in 250 g soil divided by initial J2 density (Pf/Pi) of *Meloidogyne javanica* on *Lycopersicon esculentum* (L.e.), *Dolichos lablab* (D.l.), *Elymus glaucus* (E.g.), *Sesamum indicum* (S.i.), *Crotalaria juncea* PI 207657 (PI 207657), and *Crotalaria juncea* cv. Tropic Sun (Tropic) 90 days after inoculation with an initial density (Pi) of 1.3 J2/g sand. The J2 were extracted from 250 g of soil using Baermann funnels for 3 days. Data for individual replicates (narrow bars) and means (broad bars) are presented. Plant abbreviations followed by the same letter did not have significantly different Pf/Pi indices ($P \leq 0.05$) according to the Waller–Duncan test.

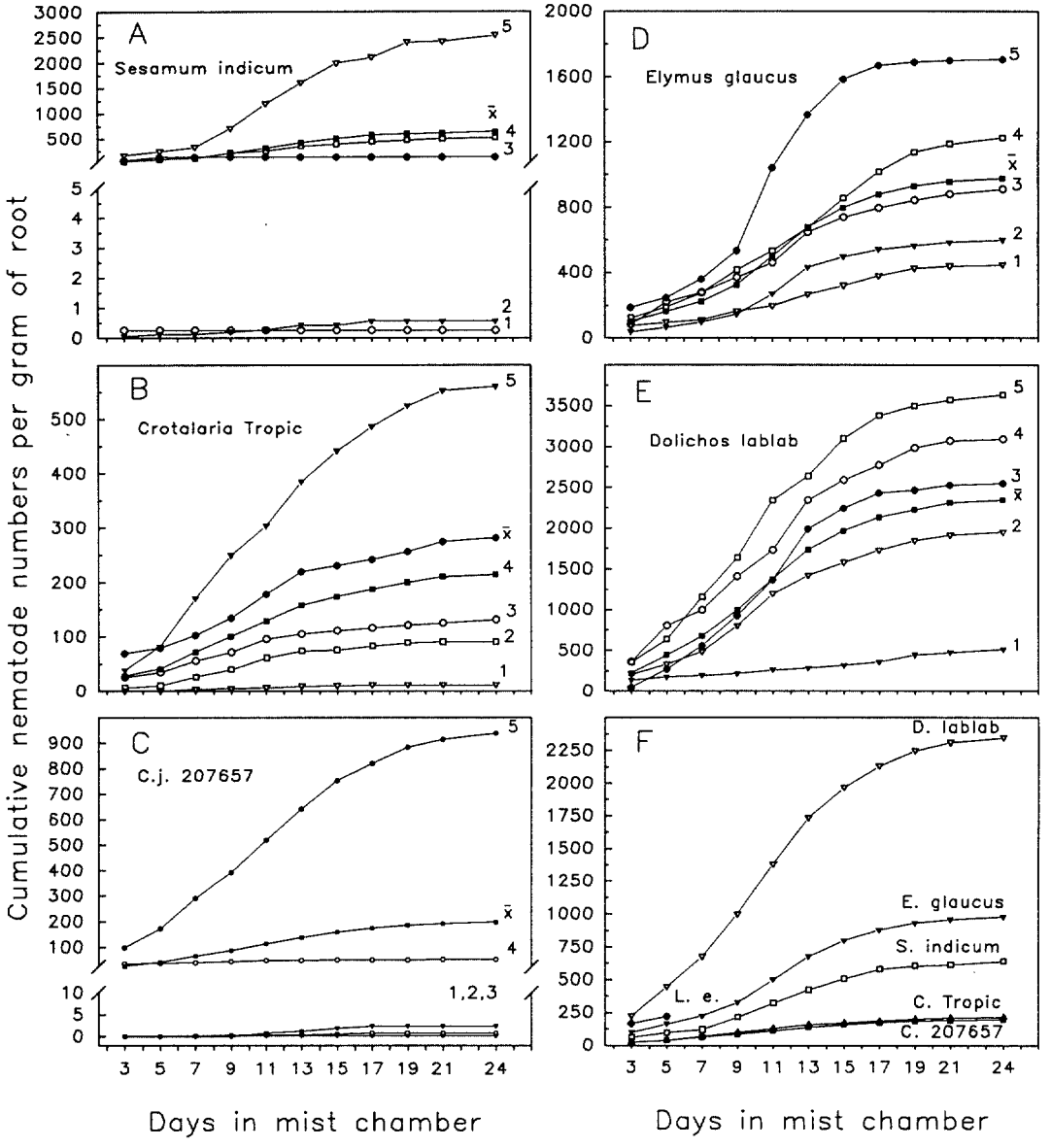

FIG. 2. Cumulative numbers of *Meloidogyne javanica* second-stage juveniles (J2) per gram of root 90 days after inoculation with an initial population density (Pi) of 1.3 J2/g sand. Nematodes were extracted from 15-g root samples over 24 days in a mist chamber. In Figs. A–E, the data for the five individual replicates and the means are presented. A) *Sesamum indicum.* B) *Crotalaria juncea* cv. Tropic Sun. (C) *Crotalaria juncea* PI 207657. D) *Elymus glaucus.* E) *Dolichos lablab.* F) Mean of five replicates from each plant (A through E).

all plants after 24 days were ca. 500% greater than the J2 numbers after 5 days.

DISCUSSION

As expected for a good host, *L. esculentum* had a high gall index and limited root growth. In this study however, the root-gall index was not an accurate parameter to access the host status of *D. lablab, E. glaucus, S. indicum,* and both *C. juncea* entries for *M. javanica.* Both *C. juncea* entries, *D. lablab, E. glaucus,* and some replicates of *S. indicum* had no galls but supported nematode reproduction, as evidenced by mist-chamber extraction of J2. The data clearly reveal that plants with a root-gall index of zero supported nematode reproduction,

TABLE 2. Cumulative *Meloidogyne javanica* juvenile (J2) numbers per gram of root for *Dolichos lablab, Lycopersicon esculentum, Elymus glaucus, Sesamum indicum, Crotalaria juncea* PI 207657, and cv. Tropic Sun.†

Plant	Cumulative second-stage juveniles at each collection period (in days)									
	3‡	5	7	9	11	13	15	17	19	21	24
D. lablab	224.8 a	447.8 a	679.1 a	999.8 a	1,381.7 a	1,737.4 a	1,968.3 a	2,134.2 a	2,248.8 a	2,311.9 a	2,346.8 a
L. esculen.	197.1 a	223.3 ab									
E. glaucus	102.8 ab	165.5 ab	226.1 ab	327.3 ab	501.4 ab	678.5 ab	800.5 ab	881.7 ab	932.9 ab	959.8 ab	978.8 ab
S. indicum	63.7 bc	99.6 bc	121.3 bc	215.9 abc	322.5 abc	423.8 abc	508.7 abc	581.4 abc	607.5 abc	615.5 abc	641.7 abc
C. j. Tropic	28.9 bc	41.9 bc	72.6 bc	100.9 bc	129.6 bc	158.9 bc	174.4 bc	188.4 bc	201.1 bc	211.7 bc	215.8 bc
C. j. PI 207657	26.9 c	42.3 c	67.0 c	88.1 c	114.3 c	139.2 c	161.4 c	175.3 c	187.9 c	194.2 c	199.2 c

† Nematodes were extracted from 15-g root samples in a mist chamber for 24 days; data are means of five replicates.
‡ Means within a column followed by the same letter are not significantly different according to the Waller–Duncan *k*-ratio *t* test ($P < 0.05$).

so a gall index must be interpreted with care.

Although the soil Pf/Pi index allowed discrimination of the host status of *L. esculentum* relative to the other plants, it did not allow differentiation of the relative host status to *M. javanica* among the other plants. The Pf/Pi index must be used with care because Pf is a function of the time at which Pf is measured.

Mist-chamber extraction of J2 was the best parameter to access reproduction of *M. javanica* on the test plants. We estimate the relative host status of the plants using mean cumulative nematode numbers per gram of root as *D. lablab, L. esculentum* > *E. glaucus, S. indicum* > both entries of *C. juncea,* in order of decreasing host suitability. The nematode numbers increased over time during mist chamber extraction, suggesting egg hatch over time. Again, the nematode numbers observed depend on the time allowed for collection. Variation in nematode reproduction among individual replicates was observed (Fig. 2), which was probably the result of genetic variability among individual plants. This is important because genetic variability for host status in seed lots of these plants must be considered in their use for nematode management.

Nematode reproduction measured by J2 numbers per gram of root was used to categorize resistance with a scale developed by McKenry (unpubl.). Plants were categorized as resistant (<0.2 nematodes per gram root), moderately resistant (0.21 to 0.5), slightly susceptible (0.51 to 2.0), and susceptible (>2.1 nematodes per gram root). Accordingly, two replicates of *C. juncea* cv. Tropic Sun, one of *C. juncea* PI 207657, and one of *S. indicum* were resistant, one replicate of *S. indicum* was moderately resistant, one replicate of *C. juncea* cv. Tropic Sun was slightly susceptible, and the remaining replicates of the test plants were susceptible (Fig. 2).

The host status and reproductive potential of *M. javanica* on *D. lablab, E. glaucus, S. indicum,* and both *C. juncea* was most accurately assessed by the number of J2 per

gram of root extracted in a mist chamber using periods longer than 5 days. The use of a root-gall index, or Pf/Pi based on soil J2, underestimated the reproductive potential of *M. javanica* on the test plants. These observations demonstrate that assessment of nematode resistance can be influenced by the time of sampling, the nematode extraction technique, and the duration of extraction used.

The reduced penetration (1) and reproduction in both *C. juncea* and some replicates of *S. indicum* may be useful in decreasing *M. javanica* numbers in infested soils, and may explain other observations wherein *Crotalaria* species have suppressed *Meloidogyne* species (12–19) or other nematode genera (3), and *S. indicum* has reduced numbers of *Meloidogyne* spp. (13–15,18). Both *Crotalaria* and *S. indicum* have potential use as cover crops to reduce *M. javanica* numbers.

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