Predicting Damage of *Meloidogyne incognita* on Watermelon

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Abstract: Quantitative growth response of watermelon (*Citrullus lanatus*) sensitive to *Meloidogyne incognita* is poorly understood. Determination of soil population densities of second-stage juveniles (J2) of *M. incognita* with Baermann funnel extraction often is inaccurate at low soil temperatures. In greenhouse experiments, three sandy soils were inoculated with dilution series of population densities of eggs or J2 of *M. incognita* and planted in small containers to watermelon 'Royal Sweet' or subjected to Baermann funnel extraction. After five weeks of incubation in the greenhouse bioassay plants in egg-inoculated soils, gall numbers on watermelon roots related more closely to inoculated population densities than J2 counts after Baermann funnel extraction. In April 2004, perpendicularly-inserted tubes (45-cm diameter, 55-cm deep) served as microplots where two methyl bromide-fumigated sandy soils were inoculated with egg suspensions of *M. incognita* at 0, 100, 1,000 or 10,000 eggs/100 cm^3 of soil in 15-cm depth. At transplanting of 4-week old watermelon seedlings, soils were sampled for the bioassay or for extraction of J2 by Baermann funnel. In the Seinhorst function of harvested biomass in relation to nematode numbers, decline of biomass with increasing population densities of *M. incognita* was accurately modeled by the inoculated eggs ($R^2 = 0.93$) and by the counts of galls on the bioassay roots ($R^2 = 0.98$); but poorly by J2 counts ($R^2 = 0.68$). Threshold levels of watermelon top dry weight to *M. incognita* were 122 eggs/100 cm^3 soil, 1.6 galls on bioassay roots, or 3.6 J2/100 cm^3 of soil. Using the bioassay in early spring for predicting risk of nematode damage appeared useful in integrated pest management systems of watermelon.

Key words: bioassay, root-knot nematode, Seinhorst function, threshold level.

Southern root-knot nematodes, *Meloidogyne incognita,* are a threat to vegetable and field crops worldwide (Sasser, 1980; Sikora and Fernandez, 2005). Despite the presence of some resistance to root-knot nematodes in cucurbits (Boyhan et al., 2003; Thies and Levi, 2007), currently no commercial watermelon (*Citrullus lanatus*) cultivars with high levels of resistance to *M. incognita* are available. In contrast, most cultivars are highly susceptible (Montalvo and Esnard, 1994). Watermelon can be substantially damaged by *M. incognita* (Davis, 2007; Thies et al., 2010), but its quantitative growth response to infection by the nematodes at different infestation levels is poorly understood. In many nematode-plant systems, yield losses are primarily influenced by pre-plant nematode population densities in soil (Ferris, 1981). Nematode damage is described by the curvilinear Seinhorst function (Seinhorst, 1970), in which plant yields are plotted against initial nematode population densities (*Pi*). A plateau describes the maximum plant yield to a specific value (i.e. the threshold level) of the nematode population density that causes plant damage. Beyond the threshold level, plant yields decrease exponentially to the minimum that may still be attainable at high population densities of the nematode pest. Thus, integrated pest management systems depend on accurate determination of initial nematode population densities to make planting decisions (Seinhorst, 1965, 1970). Soil sampling is the first step when assessing the risk of nematode damage. For *Meloidogyne* spp., initial population densities are often determined by extracting second-stage juveniles (J2) from pre-plant soil samples (Hooper, 1986). It is often necessary to examine population densities immediately before planting because a sampling long before planting, e.g., in the preceding fall of a spring planted crop, would not consider variable winter survival rates as was found for *M. incognita* (Ferris, 1985).

The Baermann funnel method, in which the migratory stages of various nematodes are extracted, requires optimum timing of sampling to obtain meaningful estimates of nematode densities in soil. For *M. incognita* with limited activity under adverse conditions (e.g. low soil temperature), quantifying the abundance of J2 in soil can be cumbersome because extraction methods primarily detect active J2 at the time of soil collection, thus often underestimate the infestation of a site (Belair, 1998; Gugino et al., 2008). Bioassays have been used in various crops for improved accuracy of determination of population densities of *Meloidogyne* spp. (Castillo et al., 2001; Gugino et al., 2008; Singh and Gaur, 1994).

In southern Indiana, watermelon seedlings are raised in greenhouses in late March to early April and transplanted to production fields in mid-April to early May when soil temperatures approximate 15 °C (Fig. 1). In the main watermelon producing area of Indiana, fields are often infested with *M. incognita* (Kruger et al., 2007), and severe yield loss or crop failure can occur due to nematode infestation (Westphal unpublished). Determining the initial population densities of the nematodes in production fields would preferentially occur during late winter/early spring to account for potential die-off during winter. Extension professionals or consultants prefer extractions of nematodes with Baermann funnel because of the ease of handling compared to more resource-intensive centrifugation methods. But this extraction method depends on the active migration of the J2 from soil. Even if the pre-planting *M. incognita*
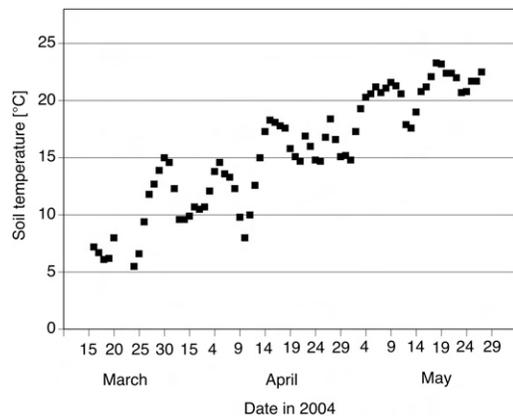
FIG. 1. Soil temperatures at 10-cm deep in bare soil in southern Indiana in early spring of 2004; the graph was generated using data for the Southwest Purdue Agricultural Centre from the web site of the Indiana State Climate Office (http://iclimate.org/).

population densities exceed threshold levels during critical soil sampling times in early spring when soils are cold, it is difficult to accurately detect critical pre-season J2 populations. An effective and simple detection method for predicting *M. incognita* population density in soil before planting is urgently needed for forecasting the potential damage of *M. incognita* on watermelon.

The objectives of this project were (A) to compare the capacity of a greenhouse bioassay with watermelon and Baermann funnel extraction of J2 to quantify known *M. incognita* population densities in soil, (B) to measure the quantitative growth response of watermelon to infestations with *M. incognita*, and (C) to determine the damage threshold level of *M. incognita* on watermelon in southern Indiana.

MATERIAL AND METHODS

A culture of *Meloidogyne incognita* originally isolated from watermelon in southern Indiana was derived from a single egg-mass (Westphal unpublished), and reared on tomato (*Solanum lycopersicum*) 'Rutgers' in the greenhouse. Nematode eggs were harvested using the NaOCl method (Hussey and Barker, 1973). Juveniles (J2) were collected with a modified Baermann funnel method, similar to that described by Hooper (1986). Seeds or seedlings of watermelon 'Royal Sweet', a common cultivar grown near Vincennes, IN were used in all greenhouse experiments and the microplot trial. Baermann funnel extraction of J2 assay and a greenhouse bioassay with watermelon were used for determining population densities of *M. incognita* in infested soil.

Greenhouse bioassay with watermelon: Plastic cone containers (20.8 cm deep and 4-cm diam.; Stuewe and Sons, Inc., Tangent, OR) were filled with 120 cm^3 of infested test soil to a depth of 1 cm below the rim of the container. Two watermelon seeds were planted 0.5-cm deep into each container. In some experiments to protect the emerging seedling from damping-off, the contents of

the containers were drenched with 50 ppm 47.6% metalaxyl (R)-2-[(2,6-dimethyl phenyl)-methoxy acetyl amino]-propionic acid methyl ester (Syngenta Crop Protection, Greensboro, NC, U.S.) and 150 ppm 50.0% fludioxonil (Syngenta Crop Protection). Containers were covered individually with parafilm (Pechiney Plastic Packaging Co., Chicago, IL). The treated containers were arranged in randomized complete block designs with sufficient spacing between containers in commercial support racks. After germination, the parafilm was removed, and seedlings thinned to one per container. Plants were watered twice daily with a sprinkling can with care being taken to avoid cross-contamination. Once a week, the units were fertilized with commercial nutrient solution (Miracle Gro All Purpose Plant Food, 15% N, 13.2% P, 12.5% K plus micronutrients, The Scotts Company LLC, Marysville, OH) at a standard concentration (10 g/3.79 L of water) needed for optimum plant growth. After five weeks in the greenhouse with 16/8 h day/night cycle at 25 °C, the tops were removed, and the roots carefully washed free of soil under tap water, weighed, and *M. incognita*-induced galls were counted.

Laboratory inoculation experiments with eggs or J2 of M. incognita: In inoculation experiments, three sandy soils including the two soils used in the microplot experiment (see below) were autoclaved twice with 24 hr between autoclavings. Infestation levels of 0, 100, 1,000, and 10,000 eggs or 0, 20, 200, and 2,000 J2/100 cm^3 of soil were established in these soils. The respective suspensions of eggs or J2 were added to 1,000 cm^3 portions of each of the test soils to accommodate the four treatments of egg inoculations and the four treatments of J2 inoculations. Four subsamples (120 cm^3 each) from each inoculated soil mix were dispensed into the watermelon greenhouse bioassay containers, and four subsamples (50 cm^3 each) were placed on modified Baermann funnels resulting in four replications per treatment per experiment. The bioassay cones were arranged in randomized complete block designs, and incubated in the greenhouse for five weeks before counting of nematode-induced galls under a magnifying glass. For Baermann funnel assays, nematodes were extracted from soil for four days in the laboratory at 21 °C, and egressed J2 were enumerated under a microscope.

Microplot experiment: At the South West Purdue Agricultural Center, Vincennes, IN sandy soils (70% sand, 20% silt, 10% clay, pH 7.0, 1.2% organic matter (OM) and 72% sand, 18% silt, 10% clay, pH 6.7, 1.3% OM) from two representative watermelon fields were placed in 45-cm-diam. by 55-cm deep polyethylene tubes (N12, Advanced Drainage Systems Inc., Hilliard, OH) inserted perpendicularly in the ground to serve as microplots. The soils used in the microplot experiment were typical for watermelon production in southern Indiana (Westphal et al., 2011). On 16 April 2004, methyl bromide was applied at 390 kg/ha as described previously (Westphal et al., 2011). Plots were immediately

covered with 0.1-mm thick, black polyethylene sheets and sealed. On 22 April 2004, the plastic tarps were cut to allow for aeration, and then removed.

On 5 May 2004, egg suspensions of greenhouse-raised *M. incognita* in 50 ml of dilute agar-water suspension (0.13% agar) were dispensed into 10 individual 7.6-cm deep holes to deliver infestation levels equivalent to 100, 1,000, and 10,000 *M. incognita* eggs/100 cm^3 of soil in the upper 15 cm depth. The same amount of dilute agar-water suspension without *M. incognita* eggs was added to the non-*M. incognita* infested plots. Each treatment was applied to both soils in four replications for a total of 32 plots. After inoculation, the top 15 cm of soil was mixed in each plot to establish a uniform infestation of *M. incognita*. On 14 May 2004, three one-month old watermelon seedlings were transplanted into each plot in an equidistant pattern for a full season crop. At the same time, multiple soil samples from the inoculation layer were collected, composited, and used for the greenhouse bioassay or for extraction of J2 by Baermann funnel (as described above). Each transplant was watered with 100 ml of nutrient solution (10 g of Miracle Gro per 3.79 L of water, 15% N, 13.2% P, 12.5% K and micro-nutrients; The Scotts Company LLC, Marysville, OH). At transplanting, plots were fertilized with commercial N-P-K fertilizer and potassium chloride delivering 112 kg N, 49 kg P, and 186 kg K per ha. Watermelon plants were maintained under standard agricultural conditions following a standard fungicide and insecticide program (Foster et al., 2002). On 11 June, one of the three watermelon seedlings was randomly chosen for excavation and evaluation of root galling severity on the scale by Bridge and Page (1980). The vines of the other two watermelon plants were trellised around two pairs of stakes adjacent to each plant in the individual plots. At harvest on 10 August 2004, fresh fruits were counted and weighed, plant tops were severed at the soil line and oven dried for dry weight determination, and roots were excavated for root rating evaluations. Soil samples consisting of multiple 2.5-cm diam. 30-cm deep soil cores per plot were taken at mid-season (11 June) and harvest (10 August) of the crop for Baermann funnel extractions of J2.

Data collection and analysis: After separate ANOVA of each of the laboratory experiments and comparison of the homogeneity of error variances the two experiments were combined. In the combined ANOVA, no soil × inoculation level interaction was found and thus the data were averaged across soils. The nematode numbers from each run of the experiment were standardized to percentages of the maximum value within the individual experiment set at 100%. Based on comments by Ostle (1954) regarding combining several groups for regression analysis, the two runs of the greenhouse inoculation experiments were combined for regression analysis. Slopes of the linear regression

lines of the detected nematodes relative to the maximum nematode density plotted against initial inoculation levels were compared by the method outlined in Steel and Torrie (1980).

Soil temperature data (at 10-cm depth of bare soil) at the Southwest Purdue Agricultural Centre were obtained from the website (http://iclimate.org/), and plotted. The microplot data were subjected to an analysis of variance (ANOVA) using soil and infestation levels as factors; and to regression analysis for the means of soil × inoculum (S × I) level in SAS (SAS Institute, Cary, NC). Initial *M. incognita* egg infestation levels, J2 counts from Baermann funnel extraction, and *M. incognita*-induced gall numbers from the bioassay watermelon roots were standardized to counts/100 cm^3 of soil. Nematode data were $\log_{10}$ (x + 1) transformed, and entered together with the watermelon plant top dry weight into the Seinhorst function f(x) = m + (max-m) $z^{(x-t)}$ where f(x) = yield, *max* = maximum yield, *m* = minimum yield, *z* = a constant related to a specific crop, *x* = nematode population density, and *t* = threshold level for nematode damage, using the software program "Seinfit" that returned the components of the function along with determination coefficient (R^2) and the sums of squares (*SS*) (Seinhorst, 1965, 1970; Viaene et al., 1997). This program is based on the double derivative method by Ferris et al. (1981). Root rating data were arcsine ($\sqrt{\text{(root rate/10)}}$) transformed prior to analysis.

Results

Laboratory inoculation experiments with eggs or J2 of M. incognita: Averaged across the two experiments and soils, maximum gall counts ranged from 86 to 118 galls/100 cm^3 of soil in egg inoculated soils, and 43 to 90 galls/100 cm^3 when J2 inocula were used (data not shown). Maximum J2 counts were 63 to 104 J2/100 cm^3 of soil following egg inoculation and 173 to 218 J2/100 cm^3 when J2 inocula were used (data not shown). When standardizing to the log-transformed maxima, and when J2 were used as inocula, quantitative detection of *M. incognita* either as galls in the bioassay (f(x) = 23.0 x - 5.4, R^2 = 0.82, P < 0.01) or extracted J2 (f(x) = 23.0 x - 1.8, R^2 = 0.81, P < 0.01) was similar sharing the same linear regression line slope of 23.0 (Fig. 2). When eggs were used as inocula, the quantitative response measured as galls in the bioassay (f(x) = 22.7 x - 4.0, R^2 = 0.84, P < 0.01) was stronger than that measured as extracted J2 (f(x) = 15.1 x - 3.5, R^2 = 0.69, P < 0.01); the fit of the data of the gall detection was characterized by a higher R^2 (Fig. 2). Linear regression lines of J2 inocula detected as galls, egg inocula detected as galls, and J2 inocula detected as J2 shared similar regression coefficients (23.0, 22.7, and 23.0, respectively) and R^2 values (0.82, 0.84, and 0.81, respectively).

Microplot experiment: Detected number of galls on bioassay roots and extracted number of J2 from the
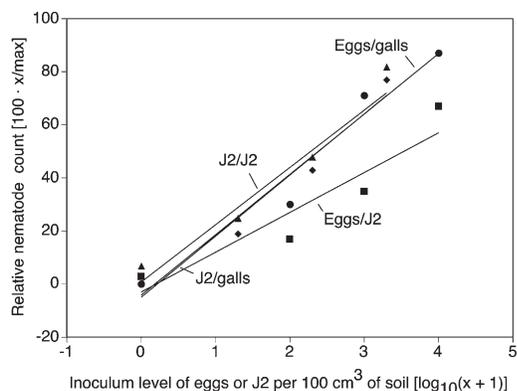
FIG. 2. Population densities of *Meloidogyne incognita* in sandy soils inoculated with either eggs or J2, and detection of J2 extracted by Baermann funnel or galls on watermelon roots in a greenhouse bioassay. Inoculation source/detection method are expressed as linear regressions (■) Eggs/J2: $f(x) = 15.1x - 3.5$; $R^2 = 0.69$; $P < 0.01$; (●) Eggs/galls: $f(x) = 22.7x - 4.0$; $R^2 = 0.84$; $P < 0.01$; (▲) J2/J2: $(x) = 23.0x - 1.8$; $R^2 = 0.81$; $P < 0.01$; (◆) J2/galls: $f(x) = 23.0x - 5.4$; $R^2 = 0.82$; $P < 0.01$.

funnels increased in response to increasing infestation levels of eggs in soil (Fig. 3). The R^2 value of the linear regression fit of the log-transformed gall counts (0.85) was higher than that of log-transformed J2 numbers (0.49). In addition, the confidence intervals at 95% were much wider for the J2 detection than for the gall detection ($P < 0.05$ versus $P < 0.01$, Fig. 3). At lower population densities of eggs inoculated in soil, galls on bioassay roots were detected (Fig. 3A) whereas no J2 were found at these same inoculation levels (Fig. 3B).

In the Seinhorst function, the watermelon top dry weight was highly related to the initial number of eggs added to the soil ($R^2 = 0.93$, Fig. 4A), as was the relationship between *M. incognita*-induced gall number on the bioassay watermelon root and the top dry weight ($R^2 = 0.98$, Fig. 4B). Conversely, the relationship between watermelon top dry weight and J2 number extracted from soil using Baermann funnels was much less related ($R^2 = 0.68$, Fig. 4C). In the microplot experiment, soil type (S) alone and soil type x inoculum

(S × I) had no significant effects on any of the parameters measured on either sampling dates, except for the significant effects of S × I on the fruit weight ($P = 0.03$; Table 1). Densities of J2 remained near or above the detectable level during the growing season (Table 1). The initial inoculum level of *M. incognita* eggs had a significant effect ($P < 0.01$) on the J2/100 cm^3 of soil, and root galling severity on a scale of 0-10 on both sampling dates (11 June and 10 August, 2004), as well as on fruit weight in both soils (Table 1). On both sampling dates, the strong positive linear relationship ($P < 0.01$) of the egg inoculum level with J2 density in soil ($f(x) = 0.41x + 0.10$, $R^2 = 0.99$ and $f(x) = 0.63x + 0.23$, $R^2 = 0.88$, respectively) or with the root galling severity ($f(x) = 0.21x - 0.03$, $R^2 = 0.89$ and $f(x) = 0.37x + 0.05$, $R^2 = 0.97$, respectively), indicated the great potential of severe damage of *M. incognita* to watermelon production with increased initial egg inoculum densities in the soil. The root weight on 11 June 2004 was significantly impacted by the inoculum level ($P < 0.05$), with a linear regression line as $f(x) = 0.70 x + 3.40$ ($R^2 = 0.43$). With increasing inoculum levels, the fruit weight was reduced as demonstrated by the negative linear regression model $f(x) = -0.21x + 1.47$ ($R^2 = 0.66$; $P < 0.01$). A threshold level for top dry weight production of watermelon 'Royal Sweet' to *M. incognita* was estimated as Pi equal to 122 eggs/100 cm^3 of soil, 1.6 galls on bioassay roots/100 cm^3 of soil, or 3.6 J2/100 cm^3 of soil.

DISCUSSION

Our microplot experiment demonstrated a significant relationship between soil infestation levels of *M. incognita* and damage to watermelon. The negative relationship between initial egg population densities of *M. incognita* and the growth of watermelon was apparent. Our results are another example of the feasibility of relating numbers of galls on bioassay roots to soil infestations with *Meloidogne* spp. In other studies, gall numbers or indices were found to be a good predictor
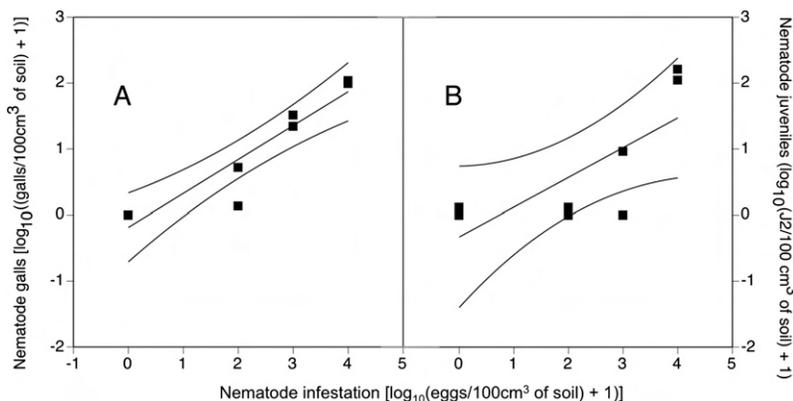

FIG. 3. Population densities of *Meloidogyne incognita* as counts of galls or J2 compared to the initial egg population densities added to field microplots, linear regression and 95% confidence intervals (A) galls on bioassay roots $f(x) = 0.51x - 0.19$; $R^2 = 0.85$; $P < 0.01$; and (B) J2 detected by Baermann funnel $f(x) = 0.45x - 0.33$; $R^2 = 0.49$; $P < 0.05$.
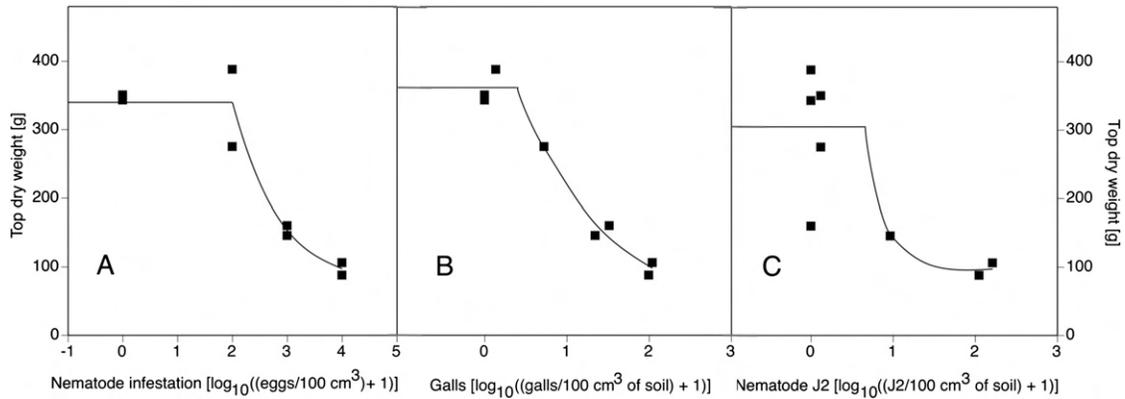
FIG. 4.   Plant top dry weights of watermelon plants in field microplots plotted over initial population densities (Pi) of *Meloidogyne incognita* described by the Seinhorst function determined by (A) egg population densities added to microplots; $f(x) = 73.68 + 265.87 \cdot 0.30^{(x - 2.00)}$ for $x >$ 2.00; $R^2 = 0.93$; $SS = 6915.25$; $m = 0.22$; $t = 2.00$; $z = 0.30$; $y_{max} = 339.55$; (B) number of *M. incognita*-induced galls on watermelon roots in a greenhouse bioassay; $f(x) = 32.48 + 328.42 \cdot 0.37^{(x - 0.41)}$ for $x > 0.41$; $R^2 = 0.98$; $SS = 1986.19$; $m = 0.09$; $t = 0.41$; $z = 0.37$; $y_{max} = 360.90$; and (C) numbers of J2 of *M. incognita* determined by Baermann funnel extraction; $f(x) = 97.20 + 206.56 \cdot 0.01^{(x - 0.66)}$ for $x > 0.66$; $R^2 = 0.68$; $SS = $ 32432.34; $m = 0.32$; $t = 0.66$; $z = 0.01$; $y_{max} = 303.76$.

of the impact of initial nematode population on growth of plants (Viaene and Abawi, 1996; Belair, 1998; Vovlas et al., 2005; Gugino et al., 2006). In our study, the Baermann funnel method was not reliable in detecting population densities of the nematode. The measured population densities of J2 in soil with Baermann funnel extraction and the known quantities present in the soil after the addition of a predetermined number of nematode eggs were poorly related. As a result, pre-plant J2 quantification from soil samples had limited predictive power for the top dry weight of watermelon in the Seinhorst model.

Comparing the results from the greenhouse studies with egg or J2 as inoculum during inoculation bioassay, it was demonstrated that the bioassay always predicted soil populations of nematodes more accurately than that of J2 counts from Baermann funnel if eggs were

used as inoculum. Similar observations were made for *Meloidogyne* spp. on rice, where the stage of the nematode at the time of soil collection impacted the efficacy of determination of the nematode population by Baermann funnel (Gaur and Sharma, 1999). Using bioassays instead of J2 counts was proposed when very low population densities of *M. arenaria* on peanut constituted the damage threshold levels (McSorley et al., 1992). This exemplified that the technique of Baermann funnel extraction was dependent on stage and population density of the nematode at the time of soil collection. In the current microplot study, the deviation of gall counts compared to J2 counts probably was impacted by the use of eggs as inocula because in the laboratory assay the quantitative response of J2 counts to inoculum level was similar when J2 were used as inocula. We have no way of predicting whether the nine

TABLE 1.   *Meloidogyne incognita* population densities and growth parameters of watermelon in two soils infested with varying egg population densities of *Meloidogyne incognita*/100 cm³ of soil in microplots at Vincennes, IN in 2004[a]

Egg inoculum level	J2 per 100 cm³ of soil[b]		Root galling [0-10][c]		Root weight [g]	Fruit weight [kg][d]	
	11 June	10 August	11 June	10 August	11 June	Soil 1	Soil 2
0	0.5 ± 0.5	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	3.8 ± 0.3	4.10 ± 0.69	7.59 ± 1.96
100	9.8 ± 1.5	118.3 ± 39.7	0.9 ± 0.1	6.0 ± 0.4	4.6 ± 0.4	3.88 ± 0.33	6.59 ± 1.18
1000	24.3 ± 5.2	247.8 ± 69.2	2.6 ± 0.3	8.5 ± 0.3	4.3 ± 0.6	4.95 ± 0.52	2.52 ± 0.86
10,000	63.5 ± 8.7	320.3 ± 56.6	6.4 ± 0.3	9.6 ± 0.3	7.2 ± 0.9	1.04 ± 0.37	1.29 ± 0.34
P_1 (soil; S)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	
P_2 (Inoculum; I)	<0.01	<0.01	<0.01	<0.01	0.0436	<0.01	
P_3 (S × I)	N.S.	N.S.	N.S.	N.S.	N.S.	0.03	
Regression							
f(x)	$0.41\ x + 0.10$	$0.63\ x + 0.23$	$0.21\ x - 0.03$	$0.37\ x + 0.05$	$0.70\ x + 3.40$	$-0.21\ x + 1.47$	
R^2	0.99	0.88	0.89	0.97	0.43	0.66	
P_{slope}; $P_{intercept}$	<0.01; 0.07	<0.01; 0.35	<0.01; 0.67	<0.01; 0.42	<0.05; <0.01	<0.01; <0.01	

[a]Probability levels were given on analysis of variance (ANOVA; $P_{1,2,3}$) and on the linear regression analysis of the various nematode and plant parameters as a function of changes in the egg inoculum levels (the linear regression lines, R^2, P_{slope} and $P_{intercept}$). Means ± standard error of the means were presented; N.S.: not significant.
[b]ANOVA was conducted after log-transformation [$\log_{10}(x + 1)$].
[c]ANOVA was conducted after arcsine-transformation [arcsine($\sqrt{(x/10)}$].
[d]ANOVA was conducted after square root-transformation [$\sqrt{(x)}$].

days of incubation from inoculation of the microplots until planting allowed sufficient juvenile hatch from the egg inocula. The bioassay was less vulnerable to differences in the prevalent nematode life stage at the time of initiation of population density determination. This makes the bioassay more versatile than the Baermann funnel in detecting nematodes in soil.

In southern Indiana, soil sampling post winter probably needs to be initiated one month before planting the seeds of commercial watermelon crop in the greenhouse, in other words, two months ahead of transplanting watermelon seedlings to the fields. Such timing would allow for making decisions in watermelon production for *M. incognita* management prior to raising seedlings in the greenhouse and transplanting to the fields. Here, we only tested soil samples at planting using the two assay methods. Because of the even more adverse conditions at an earlier sampling time we surmised that the advantages of the bioassay would increase. Information generated later would not meet growers' pre-planting needs. In integrated pest management (IPM) strategies, such early determination would still permit soil fumigation to be done before transplanting, or alternatively, selection of a non-host or resistant crop for the upcoming season without raising watermelon seedlings, therefore, averting severe yield loss of watermelon due to heavy infestations of the nematodes in production fields. Because watermelon crops in southern Indiana are often produced in rotation with soybean and maize (Westphal, 2011), alternative management tactics can be implemented. For example, some *M. incognita*-resistant soybean lines adapted to this region have been identified (Kruger et al., 2008), and their incorporation in crop sequences is proposed to mitigate the nematode problem (Westphal, 2011).

In a study on the damage threshold of *Meloidogyne hapla* on lettuce, damaging levels were independent of soil pretreatment of soil fumigation (Viane and Abawi, 1996); these levels were similar to the ones of the current study. Yet, caution needs to be exercised when extrapolating from results in fumigated soil in microplots to field situations because other pathogens in field soil may enhance plant damage, or the antagonistic potential of natural soils may reduce the damage caused by the nematodes (Sikora, 1992). Further studies of validating the information with field soils containing other potential plant pathogens of watermelon will be necessary before incorporating the current technique in practical management recommendations. We determined threshold levels of 122 eggs/100 cm^3 of soil, 1.6 galls on bioassay roots (converted on a count/100 cm^3 of soil), or 3.6 J2/100 cm^3 of soil. Even in a zero tolerance approach, the bioassay would detect these population densities reliably. In contrast, J2 populations were sometimes not detected despite later severe plant damage. These low threshold values for damage estimated in the current study were similar to the values

reported for cucurbits in warmer climates, namely *Cucumis melo* in California (Ploeg and Phillips, 2001), and watermelon in Florida (Noling, 1999).

Employing an accurate sampling and bioassay method to determine the overwintered nematode population densities is a prerequisite for appropriate and effective management of *M. incognita* on watermelon. In spring, the use of gall numbers from the greenhouse bioassay to predict the initial *M. incognita* population in cold soil was sensitive in determining pre-planting nematode population densities, and their damage potential on watermelon. The bioassay holds promise to better predicting *M. incognita* damage on watermelon, and thus improving sustainable production of this high-value crop.

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