Comparison of Reproductive Efficiency of *Meloidogyne chitwoodi* on *Solanum bulbocastanum* in Soil and In Vitro Tests

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ABSTRACT

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The reproductive efficiencies (R = final population of eggs [*Pf*]/initial population of eggs [*Pi*]) of races 1 and 2 of *Meloidogyne chitwoodi*, the Columbia root-knot nematode, were tested on 22 clones of *Solanum bulbocastanum* derived from five plant introductions. Twenty clones showed nonhost reactions ($R = <0.1$) for race 1, and 13 were poor hosts ($0.1 < R < 1.0$) for race 2. Two clones were nonhosts to both races. The host reactions of excised roots of potato, tomato, and 13 clones of *S. bulbocastanum* to races of *M. chitwoodi* were also tested in monoxenic culture. The reproductive efficiencies of both races on host genotypes in pot vs. root culture were significantly correlated. However, the two screening methods measured different levels of resistance to race 2 in the case of three clones of *S. bulbocastanum*.

Additional keywords: germ plasm enhancement

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al) is a serious pest of potato (*Solanum tuberosum* L.) (1). Infraspecific variation in host preference of certain isolates on alfalfa (*Medicago sativa* L.) and carrot (*Daucus carota* L.) has led to the identification of a second host race of *M. chitwoodi* (2,4,11). Alfalfa was thought to be a useful crop to alternate with potato to achieve some degree of control of *M. chitwoodi*, but the discovery of a second race of *M. chitwoodi* that reproduces on alfalfa has placed this in doubt. Damage in the potato cultivar Russet Burbank is characterized by galls on the outside of the tuber and necrotic spots in the flesh of peeled tubers (1), conditions that are unacceptable for processing and fresh markets. Fumigation has provided partial control, but it is clear that new cultivars with resistance would provide a more economical and environmentally sound long-term solution. Although surveys of cultivated potato germ plasm failed to identify resistance (13), a high frequency of resistance was found among accessions of *S. bulbocastanum* (13), a wild tuber-bearing relative of cultivated potato endemic to Mexico.

This paper reports on two experiments measuring resistance to *M. chitwoodi*. The first, conducted in the greenhouse, determined the reproductive efficiency (R = final populations of eggs (*Pf*)/initial inoculum (*Pi*) (8) of races 1 and 2 of *M. chitwoodi* on 22 clones selected from five different Plant Introduction (PI) accessions of *S. bulbocastanum*. The second experiment determined the host suitability of 13 *S. bulbocastanum* clones, tomato (*Lycopersicon esculentum* Mill. cv. Columbia), and potato cultivar Russet Burbank in monoxenic coculture to both races of *M. chitwoodi*, tested separately. Reproductive efficiency, which depends on the fecundity of the proportion of the primary inoculum and intermediate generations that successfully infests the host and reaches reproductive maturity, is one measure of resistance of a host crop species to *Meloidogyne* spp. (8,12).

MATERIALS AND METHODS

True seed of five PI accessions of *S. bulbocastanum* obtained from the IR-1 Potato Introduction Project, Sturgeon Bay, Wisconsin, were soaked in aqueous solution of 1.5 mg/ml of gibberellic acid 24 hr and sown in a soil mixture consisting of equal volumes of soil, peat moss, and sand. A total of 22 seedlings from five accessions were selected randomly and propagated clonally by means of stem cuttings. When approximately 7 cm tall, the plants were transplanted into 10-cm-diameter plastic pots containing loamy sand (84% sand, 10% silt, 6% clay) that had been fumigated with methyl bromide.

The nematode populations used in these experiments—WAMc1 (*M. chitwoodi*, race 1) and ORMc8 (*M. chitwoodi*, race 2)—were maintained in the Irrigated Agriculture Research and Extension Center collection (10). Inoculum was derived from single egg mass cultures and prepared by collecting eggs after shaking infested roots of tomato (cv. Columbia) in 0.5% NaOCl (3). At transplanting, an aliquot containing 5,000 eggs was pipetted onto the soil. Soil of five pots per clone was infested with each race of nematode. Pots were arranged in a completely randomized design on greenhouse tables. Potato (cv. Russet Burbank), tomato (cv. Columbia), alfalfa (cv. Thor), and pepper (*Capsicum annuum* L. cv. California Wonder) were included in all tests. Potato and tomato, excellent hosts for both races of *M. chitwoodi*, were used as standards. Alfalfa, a nonhost for race 1 but a good host for race 2, was included to detect cross-contamination between the two races. Plants were allowed to grow with regular watering and fertilization at 24 ± 3 C for 55 days. At harvest, nematode eggs were extracted by shaking roots in 0.5% aqueous NaOCl. Analysis of variance and regression analysis were performed on data transformed as ln $(x + 1)$, where x is the egg count, to correct for correlation between means and standard deviations of egg counts. Geometric means of egg counts were used to calculate R values. Mean separation was determined by Duncan's multiple range test on means of transformed egg counts, where appropriate.

Thirteen clones of *S. bulbocastanum* representing a range of R values for races 1 and 2 of *M. chitwoodi* were placed in sterile culture. This was done by excising nodes from greenhouse-grown plants, taking care to leave 15 mm of internode on apical and basal ends. These were sterilized by immersion in 5% NaOCl with 0.1% Tween 20 for 15 min, with constant stirring. Internode tissue was trimmed off and the remaining node

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(approximately 5 mm in diameter) was placed on sterile shoot growth medium containing MS salts (6), the vitamins of Nitsch and Nitsch (7), and the following (in milligrams per liter): kinetin, 0.04; indole-3-acetic acid, 0.1; gibberellic acid, 0.2; sucrose, 30,000; and agar, 6,000. Media were adjusted to pH 5.6, dispensed into 25 × 150 mm culture tubes with polypropylene caps, and autoclaved at 110 C for 15 min. Single node axillary cuttings were excised from rapidly growing cultures of potato and clones of *S. bulbocastanum* growing from nodes that were subcultured on the same medium. Cultures were grown under a 16-hr photoperiod with a photon flux of 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ from cool-white fluorescent lamps. Roots emerged from the cuttings at 6 days from subculture. Plantlets with shoots and roots required shoot growth medium and light for normal growth; a different medium was needed to culture excised roots in the absence of shoot tissue and light. After 6–10 days on shoot growth medium, root tips 0.5–1.0 cm long were excised from potato and *S. bulbocastanum* clones on shoot growth medium and from tomato seedlings germinated on sterile agar substrate. Root sections were placed on 100 × 15 mm petri dishes containing "excised root" growth medium consisting of salts of Skoog, Tsui, and White described by Orion et al (9), vitamins of Nitsch and Nitsch (7), and the following (in milligrams per liter): $NaMoO\text{-}2H_2O$, 0.25; $CuSO_4\text{-}4H_2O$, 0.25; $CoCl_2\text{-}6H_2O$. 0.025; sucrose, 20,000; agar, 15,000; and 1-naphthalene acetic acid, 0.04. The medium was adjusted to pH 4.9 and autoclaved at 110 C for 20 min before being dispensed into the petri dishes.

Freshly hatched second-stage juveniles (J2) were collected (14), freed from contamination (5), and pipetted onto the medium in petri dishes 7 days after placement of roots. Each petri dish received 200 J2. Petri dishes were sealed with Parafilm to prevent contamination and dehydration. The 30 treatments (15 host genotypes × 2 nematode races) were each replicated in five petri dishes randomized in complete blocks. Petri dishes were maintained at 21 C in the dark for 55 days before the eggs were extracted as described previously. Two uninoculated controls for each host genotype were observed during the experiment. Genotypes with R values exceeding 1.0 were designated as good hosts, those with R values between 0.1 and 1.0 as poor hosts, and those with R values less than 0.1 as nonhosts (12).

RESULTS AND DISCUSSION

Populations of the two races of *M. chitwoodi* reproduced successfully in pots on potato and tomato (R = 2.5 –28.0). Races 1 and 2 failed to reproduce on pepper (R <0.01), and race 1 failed to reproduce on alfalfa (R <0.01).

The R values of *M. chitwoodi* race 1 on *S. bulbocastanum* ranged from 0 to 12.84, with clear distinction between the two good hosts and 20 nonhosts (Table 1). In contrast, many of the responses to race 2 were intermediate. Of the 22 genotypes of *S. bulbocastanum* that were tested, two were classified as nonhosts for both races of *M. chitwoodi*. Accessions 243508 and 275184 were better hosts for race 2 (mean R values, 1.15 and 1.97, respectively) than the other three accessions. There was important variation in host suitability of *S. bulbocastanum* within species (among accessions) and within accessions (among seedlings in an accession). Single PI seed lots contained seedlings with nonhost and good host status. Since there was within-species variation in resistance phenotype, it would be necessary to use particular clones with nonhost status for both races of *M. chitwoodi* as sources of genetic resistance for potato breeding.

J2 of both races of *M. chitwoodi* infested and successfully reproduced on excised roots of potato, tomato, and certain clones of *S. bulbocastanum* in vitro (Table 2). Tomato and potato were good hosts for both races. Clones 243505.9 and 255518.6 were good hosts for race 1 in pots and in vitro culture. Five clones had R values greater than 1.0 for race 2. The quantities of eggs produced per petri dish on susceptible genotypes ranged from 1,757 to 14,867 for race 1 and from 270 to 5,019 for race 2. The correlation of the natural logarithmic means of egg number of pot culture vs. in vitro culture for race 1 (r = 0.94, P<0.001) (Fig. 1A) was highly significant. A significant correlation between pot and in vitro culture methods for race 2 was also found (r = 0.67, P<0.01) (Fig. 1B). The lower correlation coefficient for race 2 was due in part to discrepancies in the performance of three clones—243505.7, 275184.4, and 275184.7. Clone 243505.7 was defined as a good host in vitro but a nonhost in pot culture. Conversely, clones 275184.4 and 275184.7 were classified as poor hosts in vitro but good hosts in pot cul-

Table 1. Reproductive efficiency (R = Pf/Pi) of races 1 and 2 of *Meloidogyne chitwoodi* on tomato, potato, and accessions of *Solanum bulbocastanum* in pot culture

Identity	Race 1 R[x]	Race 2 R[x]
Tomato[y]	28.07 a[z]	10.41 abc
Potato[y]	7.26 abcd	2.52 abcdef
S. bulbocastanum clones derived from PI accessions:		
243505.2	<0.01 jkl	0.50 defgh
243505.4	<0.01 jklm	0.81 bcdefgh
243505.6	0.00 m	0.13 hi
243505.7	<0.01 jklm	0.02 ij
243505.9	12.84 ab	0.68 cdefgh
243508.2	<0.01 klm	0.25 efghi
243508.4	<0.01 jklm	1.50 bcdefgh
243508.6	<0.01 jklm	1.37 bcdefgh
243508.9	<0.01 jklm	1.42 bcdefgh
255518.5	<0.01 lm	0.36 efghi
255518.6	3.40 abcdef	0.19 fghi
255518.7	0.00 m	0.23 fghi
255518.8	<0.01 jklm	0.88 bcdefgh
255518.9	<0.01 jklm	0.40 efghi
275184.4	<0.01 jklm	4.19 abcde
275184.6	<0.01 jklm	0.16 ghi
275184.7	<0.01 klm	2.25 abcdefgh
275184.10	<0.01 jklm	1.26 bcdefgh
275187.1	<0.01 jklm	0.52 defgh
275187.2	<0.01 jklm	1.70 bcdefgh
275187.8	<0.01 lm	0.15 ghi
275187.10	0.00 m	<0.01 jkl

[x] R = final population (Pf)/initial population (Pi). Soil in each pot was infested with 5,000 eggs and incubated for 55 days.
[y] Tomato cv. Columbia and potato cv. Russet Burbank.
[z] Values are based on geometric means of five replicates; means within a column not sharing a common letter differ significantly (P<0.05) according to Duncan's multiple range test.

Table 2. Reproductive efficiency (R = Pf/Pi) of races 1 and 2 of *Meloidogyne chitwoodi* on excised roots of tomato, potato, and accessions of *Solanum bulbocastanum* in vitro

	Race 1		Race 2	
Identity	Number of eggs per petri dish	R[x]	Number of eggs per petri dish	R[x]
Tomato[y]	14,867.5	74.34 a[z]	5,019.1	25.10 ab
Potato[y]	4,481.8	22.41 ab	3,003.9	15.02 abc
S. bulbocastanum clones derived from PI accessions:				
243505.2	1.2	0.01 j	493.7	2.47 bcdef
243505.7	8.3	0.04 hij	270.8	1.35 cdefg
243505.9	4,486.3	22.43 ab	714.5	3.57 bcd
243508.2	27.0	0.13 ghij	34.8	0.17 fghi
243508.9	1.3	0.01 ij	2,624.4	13.12 abc
255518.6	1,757.1	8.79 abcd	53.6	0.27 efgh
255518.8	24.2	0.12 ghij	676.9	3.38 bcde
255518.9	1.6	0.01 ij	136.7	0.68 defgh
275184.4	1.8	0.01 ij	120.5	0.60 efgh
275184.7	1.6	0.01 ij	62.4	0.31 efgh
275187.1	10.4	0.05 hij	16.0	0.13 ghij
275187.8	14.9	0.07 hij	28.0	0.14 ghij
275187.10	1.5	0.01 ij	1.3	0.01 ij

[x] R = final population (Pf)/initial population (Pi). Petri dishes with 200 eggs each were incubated for 55 days.
[y] Tomato cv. Columbia and potato cv. Russet Burbank.
[z] Values are based on geometric means of five replicates; means within a column not sharing a common letter differ significantly (P <0.05) according to Duncan's multiple range test.

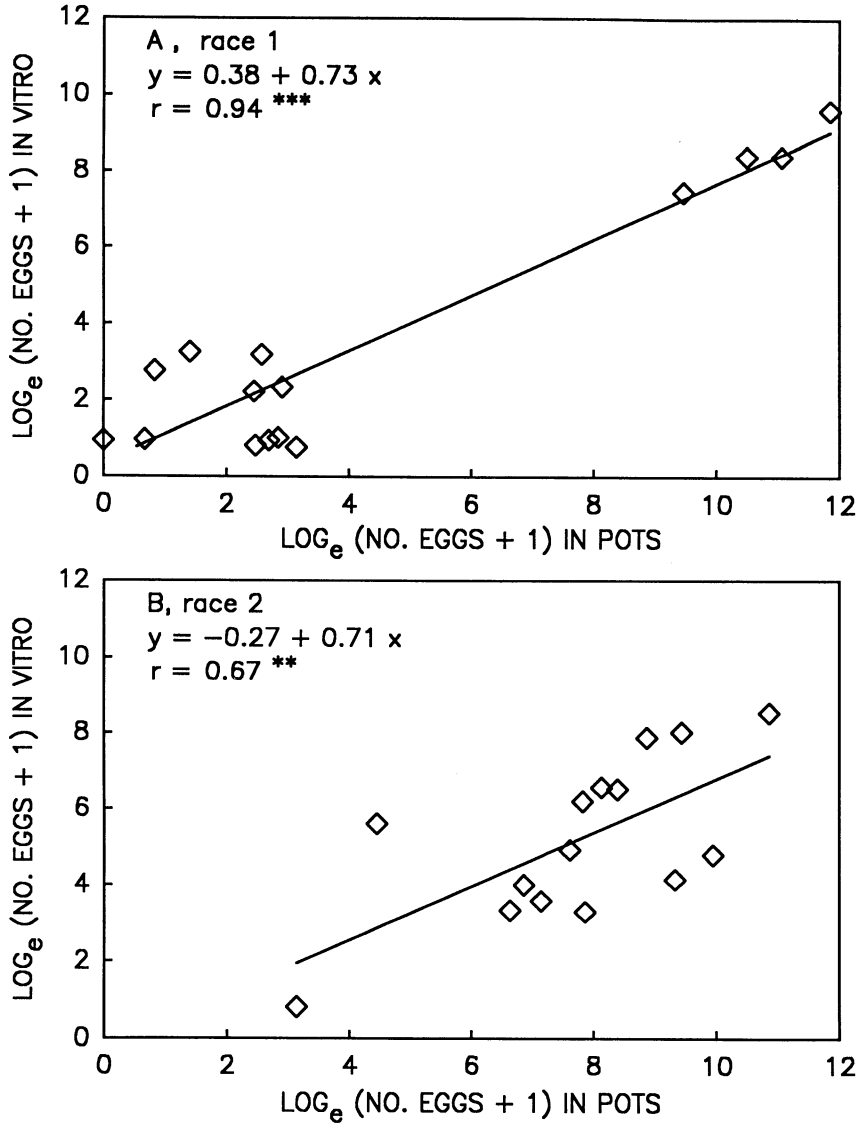

Fig. 1. Correlation of transformed egg numbers [ln $(x + 1)$] from pot and in vitro coculture for tomato, potato, and 13 accessions of *Solanum bulbocastanum*: **(A)** race 1 and **(B)** race 2.

ture. Coefficients of determination (r^2) indicated the relative proportion of total variation explained by covariation of pot vs. in vitro egg count values. The r^2 of 0.88 for race 1 and 0.45 for race 2 indicated a substantially more reliable agreement of pot vs. in vitro reproductive efficiency for race 1 than for race 2.

There was sufficient correspondence between screening methods for race 1 to promote the use of in vitro screening to identify resistance in a breeding program. The lack of concordance for race 2 detracts from this view. *M. chitwoodi* does not incite sizable root galling in most hosts. No differences in root morphology, which could be easily inspected in petri dishes, were seen in good host vs. poor host in vitro cultures. As a result, determination of *R* factors by egg counts was the only criterion usable for host response.

Reproduction of both races of nematode on potato and tomato sterile culture was high enough that this method would be an acceptable means of producing axenic inoculum of *M. chitwoodi* while avoiding the labor involved with axenization developed by Moody et al (5). It appeared that the J2 larvae successfully colonized the excised roots in a consistent fashion. It has been shown also that pure cultures of both races of *M. chitwoodi* can be maintained in vitro on excised roots.

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