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Characterization of Resistance in a Somatic Hybrid of *Solanum bulbocastanum* and *S. tuberosum* to *Meloidogyne chitwoodi*[1]

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Abstract: A somatic hybrid, CBP-233, between resistant *Solanum bulbocastanum* (SB-22) and susceptible *S. tuberosum* (R4) was tested for resistance to *Meloidogyne chitwoodi* race 1. One week after inoculation, only 0.04–0.4% of the initial inoculum (Pi, 5,000 eggs) as second stage-juveniles infected SB-22 and CBP-233 root systems, compared to 2% in R4. After 8 weeks, the number of *M. chitwoodi* in SB-22 and CBP-233 roots remained lower (0.3–1.5% of Pi) compared to R4, which increased from 2% to ca. 27%. Development of *M. chitwoodi* was delayed on SB-22 and CBP-233 by at least 2 weeks, and only half of the infective nematodes established feeding sites and matured in resistant clones compared to 99% in susceptible R4. Necrotic tissue surrounded nematodes that failed to develop in SB-22 and CBP-233. The reproductive factor (ratio of final number of eggs recovered from roots to Pi) was <0.01 for both SB-22 and CBP-233 and 46.8 for R4. Delaying inoculation of CBP-233 from 1 to 3 months after planting did not increase the chance or rate of tuber infection. Only a few *M. chitwoodi* developed to maturity on CBP-233 tubers and deposited a small number of eggs. SB-22 rarely produced tubers in these experiments, and like CBP-233 were resistant to *M. chitwoodi*. It appeared that the mechanisms of resistance to *M. chitwoodi* in roots and tubers of CBP-233 are similar.

Key words: Columbia root-knot nematode, *Meloidogyne chitwoodi*, protoplast fusion, potato, resistance, Russet Burbank, *Solanum bulbocastanum*, *Solanum tuberosum*, somatic hybrid.

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden, O'Bannon, Santo, & Finley), an important pest of potato in the Pacific Northwest (16), blemishes potato (*Solanum tuberosum* L.) tubers and reduces its value for processing or fresh market. Resistance to *M. chitwoodi* is not present in cultivated potato, and this nematode is controlled primarily by soil fumigation (13). However, resistance to *M. chitwoodi* has been identified (3) in diploid *S. bulbocastanum* Dun., a wild tuber-bearing relative of *S. tuberosum*, and transferred to the cultivated potato genome using somatic hybridization (1).

Three host races of *M. chitwoodi* have been identified: race 1 does not reproduce on alfalfa and races 2 and 3 do (9,15). Conversely, races 1 and 2 do not reproduce on *S. bulbocastanum* (4) and race 3 does (9). Preliminary observations indicated that resistance in the *S. bulbocastanum* × *S. tuberosum* somatic hybrid to *M. chitwoodi* was race specific (4). While race 1 consistently failed to sustain the original inoculum level (4), race 3 successfully parasitized different clones of the somatic hybrid (Brown et al., unpubl.). Reproduction of race 2 ranged from zero to high levels on these clones (4).

The mechanism of resistance of the somatic hybrid roots to colonization by *M. chitwoodi* race 1 is not known. Furthermore, it is not clear whether tubers of the somatic hybrid resist invasion of *M. chitwoodi* or escape infection because nematodes fail to colonize roots. The purpose of this study was to describe the nature of resistance of the somatic hybrid to *M. chitwoodi* race 1 and to test the resistance of tubers to invasion by second-stage juveniles (J2) during tuberization.

Materials and Methods

Nematode population: An isolate of *M. chitwoodi* race 1 (WAMC1) from the Washington State University Irrigated Agriculture

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Research and Extension Center root-knot nematode collection (11) was maintained on tomato, *Lycopersicon esculentum* Mill. cv. Columbian. Eggs were extracted using 0.5% NaOCl (7).

Host test plants: The nematode-resistant line of *S. bulbocastanum* PI 275187, selection no. 10 (SB-22), was identified in previous studies (1,3) from botanical seed obtained from the Potato Introduction Project, NRSP-6, Sturgeon Bay, Wisconsin. The susceptible *S. tuberosum* line PI 203900 (R4) was also obtained from the NRSP-6 project (1). Hexaploid somatic hybrid (CBP-233) (4) and protoplast donors SB-22 (diploid) and R4 (tetraploid cultivar) were cloned by stem cuttings and maintained on shoot propagation medium (2). One-month old cuttings of test plants, derived from tissue culture, were transplanted to methyl bromide-treated (0.3 kg/m^3) loamy sand soil (84% sand, 10% silt, 6% clay, <1% OM; pH 6.9) and maintained in the greenhouse (24 ± 3 C) for 3 weeks before nematode inocula were introduced. Due to unavailability of R4 plant material, tuber infection of CBP-233 was compared to that of *S. tuberosum* cv. Russet Burbank (RB) potato in two greenhouse experiments. Like R4, RB is susceptible, and its tubers are readily blemished by *M. chitwoodi* (16). Russet Burbank was propagated by planting cut tuber seed pieces.

Tomato (cv. Columbian) and alfalfa (*Medicago sativa* L. cv. Thor) were also inoculated to ascertain the viability of eggs, and possible contamination from *M. chitwoodi* race 2, respectively.

Development and reproduction: In two greenhouse tests, the number of nematodes penetrating, and their development in the roots of SB-22, R4, and CBP-233, were monitored biweekly for 8 weeks. Stem cuttings were transplanted into 10-cm-d clay pots containing loamy sand soil. Five thousand eggs (±480) in 5-ml of water were pipetted around the roots and then covered with soil. Nine roots of each plant were washed free of soil at 1, 2 (only second experiment), 4, and 6 weeks after planting, and stained with acid fuchsin (5). The stained roots were examined using a stereomicroscope, and the number of each nematode life stage counted (filiform J2, swollen J2, third- and fourth-stage juveniles; J3–J4 with hyaline peg, and pear-shaped females) at each sampling date. At 8 weeks after inoculation, roots were washed free from soil and eggs were extracted by NaOCl and counted. The reproductive factor (RF) was calculated by dividing the final egg population (Pf) by the number of eggs added (Pi) (10). In the second experiment, after extraction of eggs, roots were stained with acid fuchsin and life stages counted. The number of infective nematodes, expressed as a percentage of initial inoculum, was regressed over time (8 weeks), and the linear increase of number of nematodes in SB-22, R4, and CBP-233 roots was determined. In this paper, results of the second experiment are reported, and those differing from the first experiment are discussed.

Tuber assays in the greenhouse: Tuber infection of CBP-233 and RB was compared in two experiments. In the first experiment, 8.3 eggs/cm^3 of soil were added to each pot (600 cm^3) at 0, 30, 60, and 90 days after planting. In the second experiment, 0, 0.004, 0.04, 0.4, and 4 eggs/cm^3 of soil were added in the root zone of CBP-233 and RB plants in pots (600 cm^3) when 1,000 degree days with base temperature 5 C (DD_5) were accumulated 50 days after planting. Under field conditions, the first generation of *M. chitwoodi* develops on roots and second-generation J2 attack tubers after about 1,000 DD_5 (12). Soil temperature was monitored daily (Datapod, Omnidata International, Logan, UT) 10 cm deep. The degree of tuber damage was based on an infection index and the percentage of cull tubers. The infection index was based on ratings of 0–6, where 0 = 0, 1 = 1–3, 2 = 4–5, 3 = 6–9, 4 = 10–49, 5 = 50–99, 6 = >100 infection sites per tuber. Tubers with a rating of 3 or higher were classified as culls. The infection indices and percentages of cull tubers for the two studies were regressed against time intervals and inoculum densities, respec-

tively, to determine the relative susceptibilities of RB and CBP-233 to *M. chitwoodi.*

Tuber assays in the field: Tuber resistance of CBP-233 and R4 to *M. chitwoodi* race 1 was evaluated in field bucket microplots (14) at Prosser, Washington. Fifteen liters of field soil (81% sand, 17% silt, 2% clay, 0.9% organic matter; pH 6.9) previously treated with methyl bromide (0.3 kg/m^3) were placed in 19-liter plastic buckets and planted. Three sets of plants (five replicates/plant entry) were either inoculated at planting (18 May 1993) or at 30 and 60 days after planting. The inoculum per plant consisted of 25,000 eggs in 25 ml water, added to five holes (1.7 egg/cm^3 soil). On 7 October, plots were harvested and at least 10 tubers per bucket were hand-peeled and examined for *M. chitwoodi* infection. The suspected infection sites were excised, stained, and examined using a stereomicroscope. All pear-shaped female *M. chitwoodi* from CBP-233 and 10 from R4 tubers were excavated and measured, using a drawing tube.

Treatments of both experiments were arranged in randomized complete blocks, and field data were subjected to analysis of variance. Means were separated by Duncan's multiple-range test.

Results

Meloidogyne chitwoodi race 1 reproduced efficiently on Columbian tomato, and the reproductive factor (RF) ranged between 8 and 14 in four different experiments. For alfalfa, RF was less than 0.01.

Development and reproduction: The number of infective nematodes in R4 roots gradually increased from 2% at week 1 to ca. 27% of the initial inoculum at week 8 after inoculation ($Y = -1.09 + 3.3\,X$, $r = 0.96$, $P < 0.01$, where Y is the percentage of initial inoculum, 5,000 eggs, detected at X = number of weeks after inoculation) (Fig. 1). The corresponding numbers for SB-22 ($Y = 0.8 - 0.01\,X$, $r = -0.05$, $P = 1.0$) and CBP-233 ($Y = 1.0 - 0.05\,X$, $r = -0.2$, $P = 1.0$) remained virtually un-

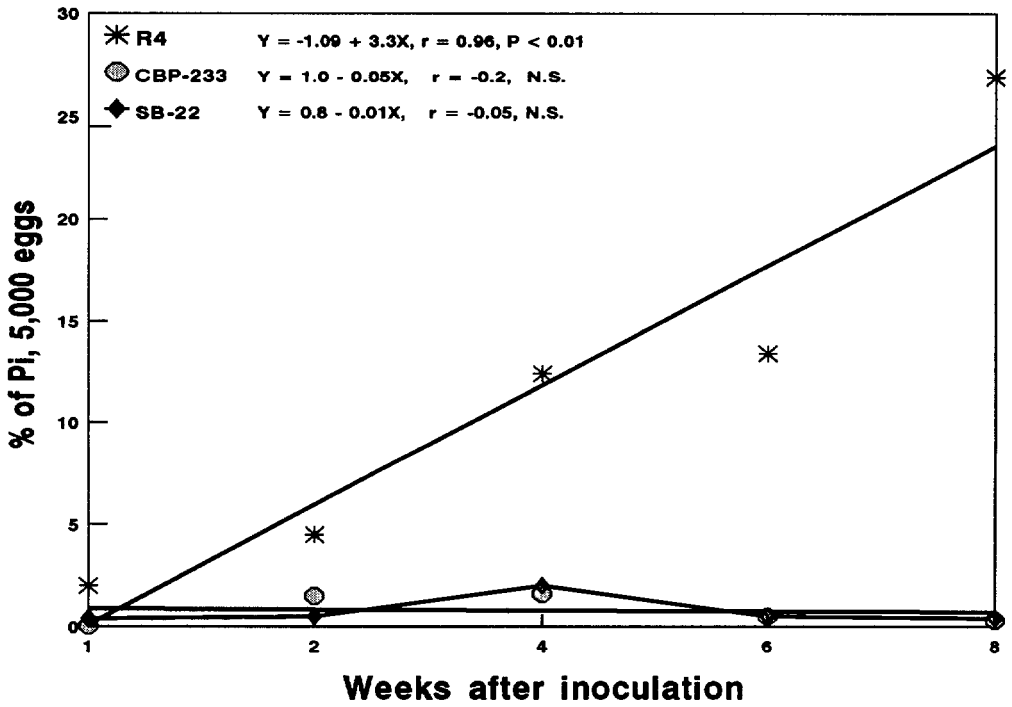

FIG. 1. A) Linear changes of *Meloidogyne chitwoodi* race 1 population in susceptible *Solanum tuberosum* (R4), resistant *S. bulbocastanum* (SB-22), and R4 × SB-22 somatic hybrid (CBP-233) after inoculation with 5,000 eggs (Pi).

TABLE 1. Development and reproduction of *Meloidogyne chitwoodi* on *Solanum tuberosum* (R4), *S. bulbocastanum* (SB-22), and their somatic hybrid (CBP-233) during 8 weeks after inoculating with 5,000 eggs (Pi).

Host	Weeks before a nematode life stage† was detected					RF‡
	J2	SJ2	J3–J4	♀	Eggs	
R4	1	2	4	4	6	46.8 a
SB-22	1	4	4	6	8	<0.01 b
CBP-233	1	4	4	6	8	<0.01 b

† Life stages of nematode consisted of filiform second-stage juveniles (J2), swollen J2 (SJ2), third- and fourth-stage juvenile with hyaline pegs (J3–J4), and pear-shaped females (♀).

‡RF = final population of eggs, Pf ÷ initial population, Pi. Eggs were extracted 8 weeks after inoculation.

changed and always lower than R4. It was noteworthy that the infection pattern of SB-22 roots was somewhat different in two experiments. In the first experiment (data not shown), a large number of nematodes entered the roots initially and then declined to 0.1% of the initial inoculum after 6 weeks. In the second experiment, the number of infective nematodes was low at the end of the first week following inoculation (Fig. 1).

Development of nematodes was slower in SB-22 and CBP-233 than R4 roots by at least 2 weeks (Table 1). Feeding juveniles (swollen J2), pear-shaped females, and eggs were first detected in R4 roots at week 2, 4, and 6, respectively. The corresponding stages of *M. chitwoodi* in SB-22 and CBP-233 roots were first detected at week 4, 6, and 8, respectively. At week 8, there were 1,343 ± 425 pear-shaped females (over 99% of infective nematodes) on R4 roots compared to 7 ± 4, and 10 ± 6 on SB-22 and CBP-233, respectively. *M. chitwoodi* reproduced abundantly on R4 with RF = 46.8 (Table 1), compared to only a few eggs on SB-22, and CBP-233 with RF < 0.01.

Many *M. chitwoodi* that penetrated CBP-233 roots failed to develop beyond the filiform J2 stage and were surrounded by necrotic tissue, whereas those in R4 roots developed normally without inciting host-tissue discoloration (Fig. 2).

Tuber assays in the greenhouse: The percentage of CBP-233 tubers damaged by *M. chitwoodi* changed very little (0–10% cull), irrespective of inoculum density or time of inoculation. The percentages of RB tubers rated as culls were directly related to inoculum density ($r = 0.92$, $P < 0.05$) (Fig. 3), and inversely to time of inoculation ($r = -0.92$, $P < 0.1$) (Fig. 4). Initial densities of 0.4 and 4 *M. chitwoodi* eggs per cm^3 soil at 1,000 DD_5 caused 30 and 60% of tuber

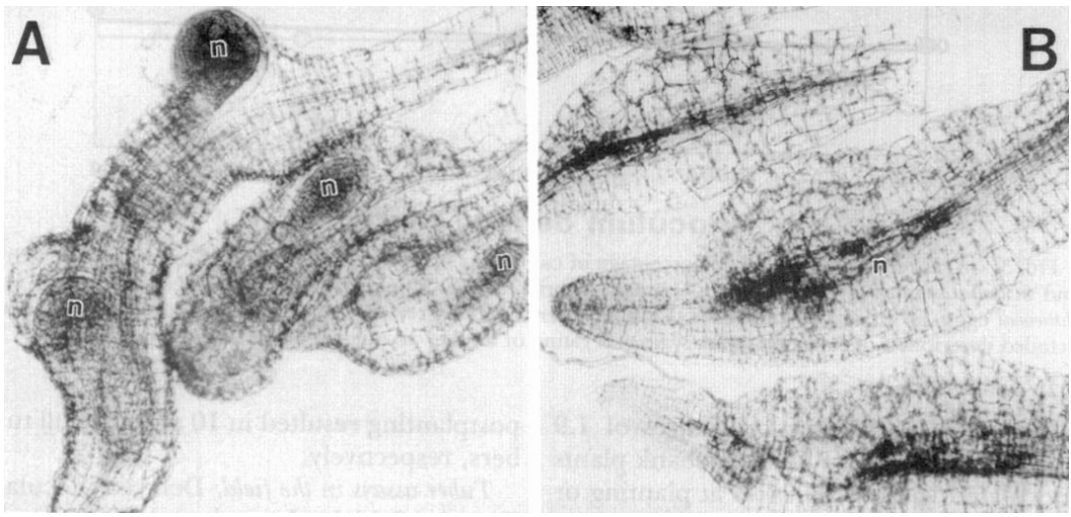

FIG. 2. A) Mature *Meloidogyne chitwoodi* race 1 female (n) in susceptible *Solanum tuberosum* (R4) and B) second-stage juveniles surrounded by necrotic tissue of resistant *S. bulbocastanum* × R4 somatic hybrid (CBP-233) 8 weeks after inoculation with eggs.

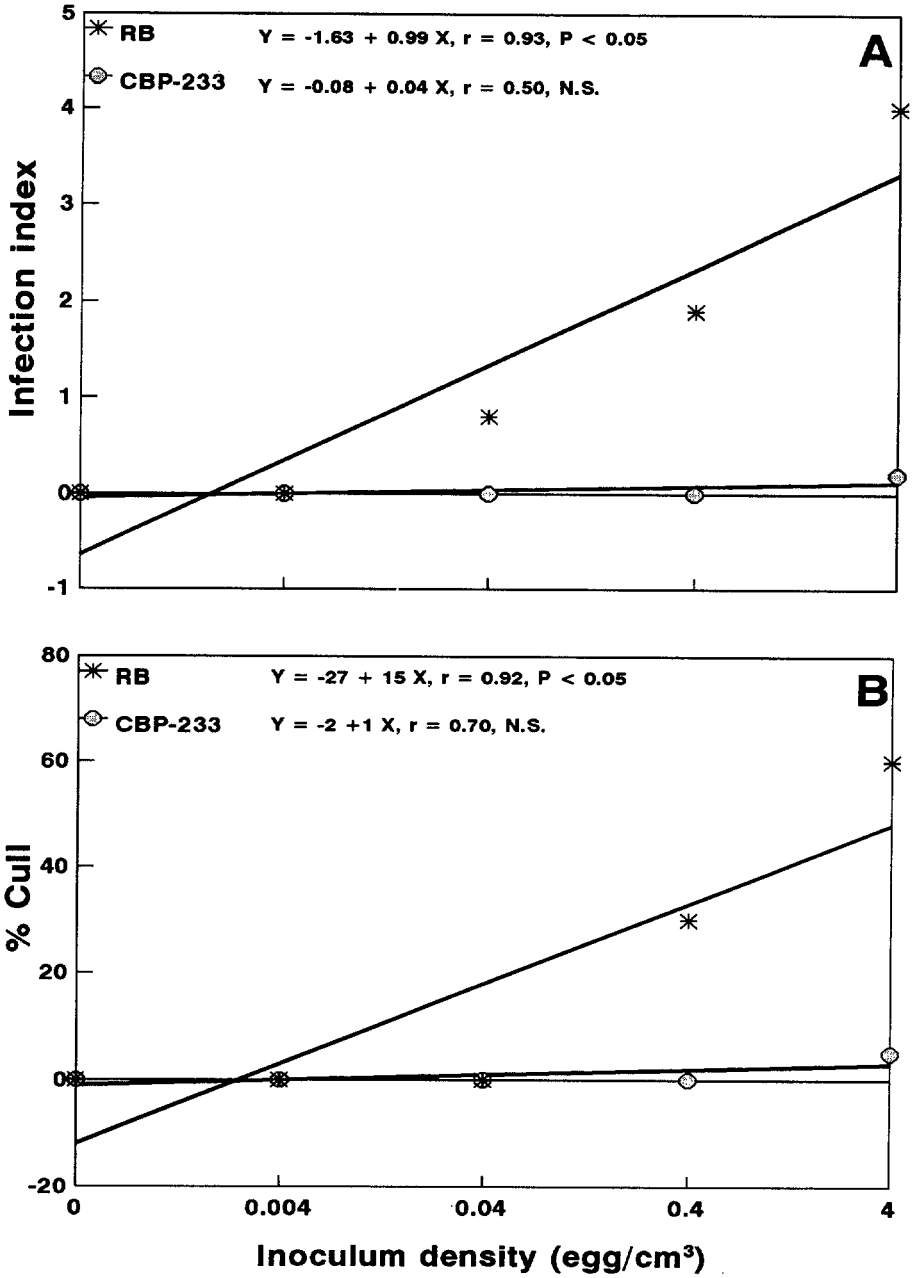

FIG. 3. A) Infection index and B) percentage of cull tubers of *Solanum tuberosum* cv. Russet Burbank (RB) and *S. bulbocastanum* × *S. tuberosum* R4 somatic hybrid (CBP-233) inoculated with different number of *M. chitwoodi* eggs/cm³ of soil 50 days after planting, when 1,000 degree days at base 5 C was accumulated. For detailed description of infection index scale and rating of a tuber as cull, see Table 2 footnote.

culls on RB with infection indices of 1.9 and 4, respectively. Russet Burbank plants inoculated with *M. chitwoodi* at planting or 30 days later produced 100% cull tubers with infection indices of 6 and 5.6, respectively. Inoculating RB at 60 and 90 days postplanting resulted in 10 and 0% cull tubers, respectively.

Tuber assays in the field: Delayed inoculations in field bucket microplots gave results similar to the greenhouse experiments. Time of inoculation did not influ-

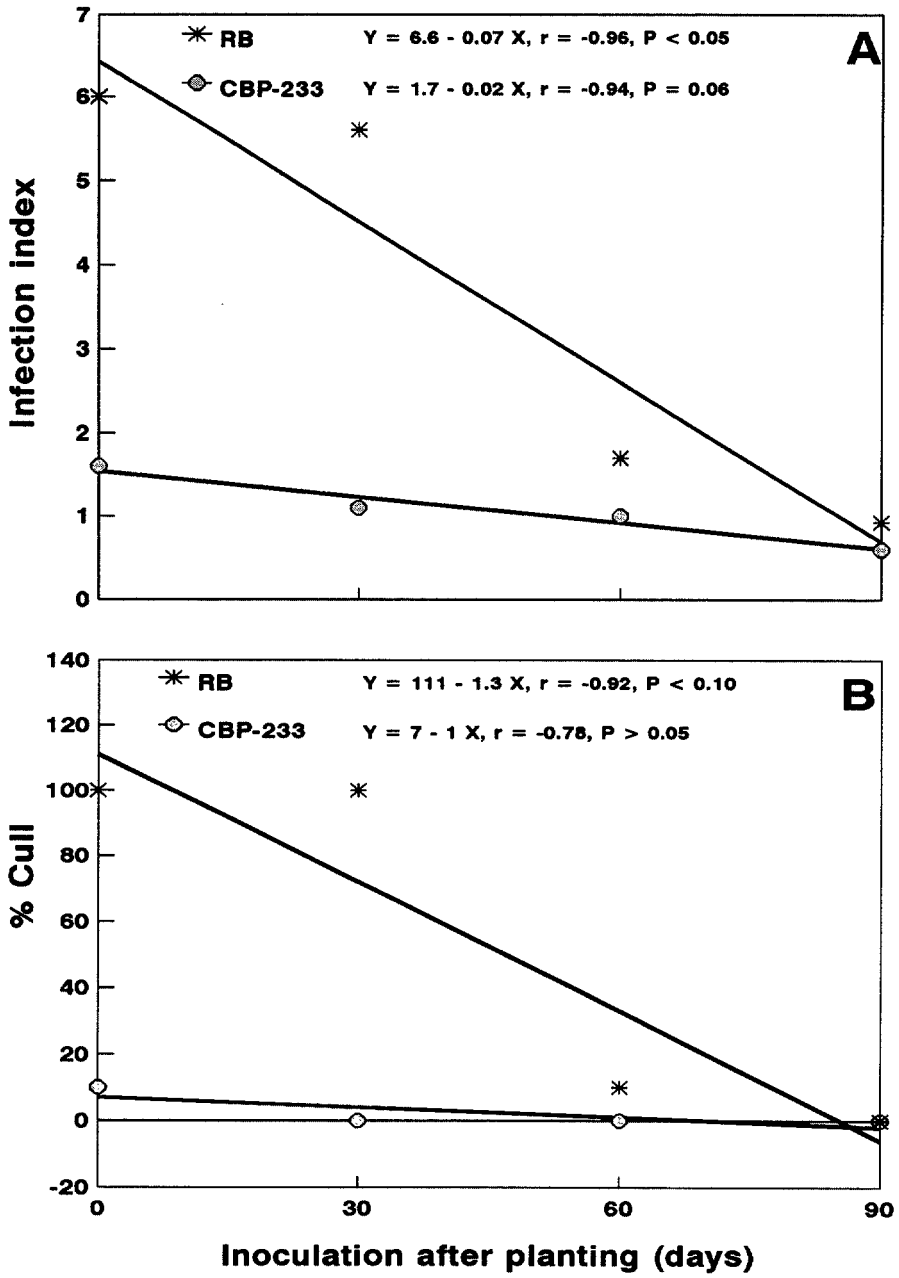

FIG. 4. A) Infection index and B) percentage of cull tubers of *Solanum tuberosum* cv. Russet Burbank (RB) and *S. bulbocastanum* × *S. tuberosum* R4 somatic hybrid (CBP-233) inoculated with 8.3 *M. chitwoodi* eggs/cm^3 of soil at different days after planting. For a detailed description of infection index scale and rating of a tuber as cull see Table 2 footnote.

ence *M. chitwoodi* damage on CBP-233 tubers (r = 0.061, NS), but it was inversely (r = −0.85, P <0.05) correlated for R4 (Table 2).

A few small, brown lesions were observed on CBP-233 tubers after peeling. Many of these lesions appeared to be failed infection sites, some harbored filiform J2, some of which developed to maturity. Mature females on CBP-233 were smaller (0.258 ± 0.03 × 0.158 ± 0.02 mm) than those of R4 (0.410 ± 0.01 × 0.258 ± 0.2

TABLE 2. Infection index and percentage of cull tubers of *Solanum tuberosum* (R4) and somatic hybrid (CBP-233) (*S. bulbocastanum* × R4) after inoculating with 1.7 *Meloidogyne chitwoodi* race 1 eggs/cm^3 soil at different times after planting in bucket microplots.

Time of inoculation (days)	Infection index¶		% cull	
	R4	CBP-233	R4	CBP-233
0	5.9 a	0.2 d	100 a	0 d
30	3.8 b	0.5 d	64 b	15 d
60	2.2 c	0.3 d	40 c	2 d

Values are the means of five replicates. Means within columns and rows followed by the same letter do not differ at $P < 0.05$ according to Duncan's multiple-range test.

¶ Infection index based on a scale of 0–6, where 0 = 0, 1 = 1–3, 2 = 4–5, 3 = 6–9, 4 = 10–49, 5 = 50–99, 6 = >100 infection sites per tuber. A tuber with infection index of 3 or more was considered a cull.

mm), and they deposited fewer eggs (exact count was not made).

DISCUSSION

Root resistance in SB-22 and CBP-233 to *M. chitwoodi* race 1 was characterized by the failure of nematodes to develop normally and deposit large number of eggs. It appeared that J2 were excluded from root penetration in the experiment depicted in Fig. 1, but in another experiment (data not shown) numerous J2 initially entered SB-22 roots. Egression of *M. chitwoodi* from SB-22 or CBP-233 roots was not closely monitored in the present studies. It is possible that *M. chitwoodi* J2 penetrated SB-22 and CBP-233 roots but exited later. A similar phenomenon occurred in alfalfa roots inoculated with race 1 of *M. chitwoodi* (8). Alfalfa is considered to be a nonhost to race 1 of *M. chitwoodi* (15).

Solanum bulbocastanum rarely produced tubers in these experiments to compare with CBP-233 tubers. Nonetheless, the few small tubers recovered were free from nematode infection. The resistance of CBP-233 tubers to *M. chitwoodi* race 1 seems to be sustained when new inoculum is added at later dates. Inoculation of potato plants 30 or more days after planting ensured that viable *M. chitwoodi* were available when CBP-233 tubers were formed. Nonetheless, these nematodes failed to establish in tubers. We believe that a possible hypersensitive reaction (i.e., darkening of the feeding sites) was mainly responsible for the failure of *M. chitwoodi* to mature normally or deposit a large number of eggs in CBP-233 tubers. The discoloration of infected tissue may have been caused by formation and (or) oxidation of phenolic compounds as suggested for certain root-knot nematodes and their incompatible hosts (6). Fortunately, the number of necrotic spots on CBP-233 tubers was minimal and did not seriously blemish the tubers. Lower levels of visible nematode damage on tubers of susceptible clones that were inoculated 30 days after planting may be due to insufficient exposure to the infectious nematodes.

A backcross program is being conducted to introduce the resistance to *M. chitwoodi* into commercially acceptable potato varieties. Resistance will be a valuable component of an integrated program that seeks to reduce or eliminate chemical inputs.

LITERATURE CITED

1. Austin, A., J. D. Pohlman, C. R. Brown, H. Mojtahedi, G. S. Santo, D. S. Douches, and J. P. Helgeson. 1993. Interspecific somatic hybridization between *Solanum tuberosum* L. and *S. bulbocastanum* Dun. as a means of transferring nematode resistance. American Potato Journal 70:485–495.

2. Brown, C. R., S. Kwiatkowski, M. W. Martin, and P. E. Martin. 1988. Eradication of PVS from potato clones through excision of meristems from *in vitro*, heat-treated shoot tips. American Potato Journal 65:633–638.

3. Brown, C. R., H. Mojtahedi, and G. S. Santo. 1989. Comparison of reproductive efficiency of *Meloidogyne chitwoodi* on *Solanum bulbocastanum* in soil and *in vitro* tests. Plant Disease 73:957–959.

4. Brown, C. R., H. Mojtahedi, and G. S. Santo. 1994. Enhancing resistance to root-knot nematodes derived from wild *Solanum* species in potato germplasm. Pp 426–38 *in* G. W. Zehnder et al., eds. Advances in potato pest management. St. Paul MN: The American Phytopathological Society Press.

5. Byrd, D. W., Jr., R. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissue for detection of nematodes. Journal of Nematology 15:142–143.

6. Huang, J. S. 1985. Mechanisms of resistance to root-knot nematodes. Pp. 165–174 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

7. Hussey, R. S., and K. R. Barker. 1973. A com-

parison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

8. Mojtahedi, H., G. S. Santo, and J. N. Pinkerton. 1988. Differential response of Thor alfalfa to *Meloidogyne chitwoodi* races and *M. hapla*. Journal of Nematology 20:410–416.

9. Mojtahedi, H., G. S. Santo, C. R. Brown, H. Ferris, and V. Williamson. 1994. A new host race of *Meloidogyne chitwoodi* from California. Plant Disease 78:1010.

10. Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mededelingen LandbouhogeSchool, Wageningen 66:3–46.

11. Pinkerton, J. N., H. Mojtahedi, and G. S. Santo. 1987. Reproductive efficiency of Pacific Northwest populations of *Meloidogyne chitwoodi* on alfalfa. Plant Disease 71:345–348.

12. Pinkerton, J. N., G. S. Santo, and H. Mojtahedi. 1991. Population dynamics of *Meloidogyne chitwoodi* on Russet Burbank potatoes in relation to degree-day accumulation. Journal of Nematology 23: 283–290.

13. Pinkerton, J. N., G. S. Santo, R. P. Ponti, and J. H. Wilson. 1986. Control of *Meloidogyne chitwoodi* in commercially grown Russet Burbank potatoes. Plant Disease 70:860–863.

14. Pinkerton, J. N., G. S. Santo, and J. H. Wilson. 1989. A bucket microplot technique for studying phytonematodes. Plant Disease 73:63–65.

15. Santo, G. S., and J. N. Pinkerton. 1985. A second race of *Meloidogyne chitwoodi* discovered in Washington. Plant Disease 69:631.

16. Strand, L. L., and P. A. Rude, eds. 1992. Integrated pest management for potatoes in the western United States. University of California, Division of Agriculture and Natural Resources, Publication 3316, USDA Western Regional Research Publication 011.