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Influence of Soybean Cultivar on Reproduction of *Heterodera glycines* in Monoxenic Culture

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Abstract: Nematodes produced in monoxenic culture are used for many research purposes. To maximize the number of *Heterodera glycines* produced in culture, 24 soybean cultivars (maturity groups 0–8) were evaluated for host suitability. A strain of *H. glycines* race 3, maintained in monoxenic culture on excised soybean root tips of cv. Kent, was inoculated into 20 petri dishes of each cultivar. The highest numbers of first-generation females per petri dish were produced on cultivars Bass, Williams 82, Kent, Proto, and Chapman, and the lowest on cultivars Lambert and Chesapeake. A diapause-like period with decreased nematode production was recorded on some cultivars but not others. Six generations of cultivation on CX 366 did not affect the number of females produced. The results indicated that soybean maturity group could not be used as a parameter for selecting the optimum cultivars for nematode production, and that only 12 petri dishes needed to be counted to determine a 60-female difference per petri dish among cultivars. This study demonstrated that *H. glycines* populations in monoxenic culture can be more than quadrupled by selection of an appropriate soybean cultivar.

Key words: *Glycine max*, *Heterodera glycines*, monoxenic culture, propagation, root tip culture, soybean, soybean cyst nematode, technique, tissue culture.

Plant-parasitic nematodes in monoxenic culture permit observations without the confounding effects of diverse soil flora and fauna, are not obscured by soil particles, and are useful for synchronizing nematode production. They also take less space and daily maintenance than greenhouse or field plants. Consequently, a majority of the agronomically important tylenchid species have been maintained in monoxenic culture.

Research applications of these cultures include studies of nematode life cycles and population dynamics (Ko et al., 1996; Rebois and Huettel, 1986); nematode reproduction on transformed roots (Kumar and Forrest, 1990); interactions among nematodes, plants, and the physical environment (Hashmi et al., 1994; Johnson and Viglierchio, 1969a, 1969b; Sudirman and Webster, 1995); and investigations with electron microscopy (Orion et al., 1980, 1995). Additionally, nematodes from monoxenic culture have been used for screening nematode resistance in plants and for looking at effects on resistance when compounds are added to the media (e.g. Fassuliotis and Bhatt, 1982; Hashmi et al., 1993; Huettel and Hammerschlag, 1986, 1993; Rivera-Smith et al., 1991) and for assays of nematode biocontrol agents (Meyer et al., 1990; Meyer and Huettel, 1996; Meyer and Meyer, 1995; Verdejo and Jaffee, 1988).

Because monoxenic cultures are useful for nematology research, the current study was done to aid in maximizing nematode production in root explant cultures. *Heterodera glycines* Ichinohe (soybean cyst nematode) was selected for the investigation because this nematode is a major pest of soybean plants worldwide and is, consequently, a focal point for a number of research projects. Quantitative data are needed on variation of female reproduction among cultivars in order to maximize nematode harvest. The purpose of this study was to determine the number of first-generation females produced on various susceptible cultivars. Beltsville Nematology Laboratory workers frequently have noticed that monoxenic cultures may experience a form of dormancy or diapause during certain months of the year (unpubl.). This has been noted with *H. glycines* in the field, where it is related to such factors as seasonal changes in soil tempera-

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ture and to host plant phenology (Schmitt, 1992; Yen et al., 1995). Potential interactive effects of cultivar selection and "diapause months" on nematode reproduction were studied to determine whether such a phenomenon could actually be measured in the laboratory, and to ascertain whether it should be taken into account during soybean cultivar selection.

Materials and Methods

The *H. glycines* race 3 isolate used for these experiments originated from soybean fields in Maryland and has been maintained in gnotobiotic cultures at the Beltsville Nematology Laboratory for more than 5 years. The nematode was cultured in sterile petri dishes containing excised soybean root tips (*Glycine max* (L.) Merr. cv. Kent) grown on Gamborg's B-5 medium. Populations of first-generation females were counted from root explant cultures of 24 soybean cultivars representing maturity groups 0–8 (Table 1). A minimum of two cultivars were tested from each maturity group. To conduct the tests, 30 to 50 seeds per cultivar were surface-sterilized with 95% EtOH for 3 minutes, followed by 0.73% sodium hypochlorite for 10 minutes. The seeds were then germinated in petri dishes containing 1.5% water agar, and 3 days later the root tips (ca. 1 cm long) were excised. Thirteen petri dishes of Gamborg's B-5 medium each received two excised soybean root tips. The roots were incubated at 28.8 °C for 2 days, and 10 gravid *H. glycines* females (each female with an attached egg mass) from monoxenic culture on cv. Kent were placed within 1–2 mm of the growing root tips on each petri dish. The cultures were incubated at 28.8 °C for 35 days in the dark (one generation), and females from 10 randomly selected petri dishes per soybean cultivar were then counted. The experiment was repeated once for each cultivar, for a total of two trials and 20 petri dishes per cultivar (exceptions: Proto, 19 petri dishes; Lambert, 19 petri dishes; Williams 82, 19 petri dishes; Crockett, 14 petri dishes). First-generation females were harvested from 13 cultivars (Table 2) as above during a "diapause" month (October, November, December, or January) and a "nondiapause" month to determine whether time of year affected population counts.

TABLE 1. Mean number of first-generation *Heterodera glycines* females produced on selected soybean cultivars in root explant cultures.

Soybean cultivar and maturity group	Females per petri dish[a]
Bass (3)	139.0 a
Williams 82 (3)	137.6 a
Kent (4)	126.5 a
Proto (0)	125.2 a
Chapman (2)	123.6 a
Braxton (7)	120.4 ab
Brim (6)	117.2 ab
Kenwood (2)	112.8 abc
Parker (1)	112.8 abc
Felix (1)	99.5 abcd
Duocrop (7)	80.6 bcde
SS 390 (3)	79.7 bcde
Crockett (8)	75.9 bcdef
CX 366 (3)	74.8 cdef
Choska (6)	73.9 def
Corsica (4)	63.5 ef
Essex (5)	59.9 ef
Johnston (8)	53.4 efg
Morgan (4)	49.9 fg
Hutcheson (5)	49.2 fg
Pioneer 9442 (4)	35.9 gh
Pioneer 9392 (3)	25.1 hi
Lambert (0)	21.8 i
Chesapeake (4)	21.7 i

[a] Least squares means followed by the same letter are not significantly different ($P \leq 0.05$), based on analysis of log-transformed data.

One cultivar, CX 366, was selected for tests to determine whether number of females produced would increase with repeated culturing on a soybean cultivar. Two trials with CX 366 were conducted with *H. glycines* produced on Kent, and three trials with CX 366 were done after five to six generations of monoxenic culture on CX 366. Nematode populations were counted from 10 petri dishes per trial (females from only four petri dishes were counted for one trial conducted after five generations of culture on CX 366).

To determine whether differences in female numbers were due to ability of roots to grow in monoxenic culture, five petri dishes of roots from each cultivar were weighed 4

TABLE 2. Mean number of first-generation *Heterodera glycines* females produced during "diapause" and "nondiapause" months on selected soybean cultivars in root explant cultures.

Soybean cultivar	Females per petri dish[a]	
	Diapause[b]	Nondiapause
Williams 82	164 a	115 cde
Parker	136 ab	93 cde
Chapman	120 ab	125 bc
Brim	120 ab	115 cde
Felix	113 abc	87 cde
Kenwood	107 bcd	119 cd
Crockett	83 bcde	80 def
Braxton[c]	76 cde	191 a
CX 366	72 de	77 ef
Morgan[c]	66 e	38 g
Johnston	50 e	57 f
SS 390[c]	49 e	129 ab
Hutcheson[c]	29 f	82 ef

[a] Least squares means within a column followed by the same letter are not significantly different ($P \leq 0.05$), based on analysis of log-transformed data.

[b] "Diapause" months were those during which root explant cultures of *H. glycines* on cv. Kent were observed to produce decreased numbers of nematodes. The months were October, November, December, and January.

[c] Difference between "diapause" period and "nondiapause" period significant at 0.05 level.

weeks after transfer of two root tips (Hashmi et al., 1993). Petri dishes were individually microwaved for 45 seconds on "high" setting and the roots were removed with forceps, rinsed in warm water, and patted dry on paper towels. Fresh weights and dry weights were recorded, the latter after 72 hr at 60 °C. Mean root weights were determined for each cultivar ($N = 10$ roots per cultivar). Exceptions were Corsica, $N = 8$; Hutcheson, $N = 6$; Pioneer 9442, $N = 9$; SS 390, $N = 8$.

In all statistical analyses, a $\log_{10}$ transformation of counts was used to correct for variance heterogeneity. The least squares means (in number of females per petri dish) and mean comparisons are reported in Tables 1–3.

To compare female populations on soybean cultivars, the number of females per petri dish was analyzed in a two-factor design using PROC MIXED (SAS Institute, Cary, NC), where "trial" was the random factor and "cultivar" was the fixed factor. Only the first two trials of CX 366, with nematodes cultured on Kent, were used.

TABLE 3. Soybean maturity group and mean number of first-generation *Heterodera glycines* females produced in root explant cultures.

Soybean maturity group (number of cultivars tested)	Females per petri dish[a]
2 (2)	118.1 a
1 (2)	106.0 a
7 (2)	98.5 ab
6 (2)	93.0 ab
3 (5)	77.5 bc
8 (2)	61.7 cd
5 (2)	54.3 d
0 (2)	52.5 d
4 (5)	50.1 d

[a] Least squares means followed by the same letter are not significantly different ($P \leq 0.05$), based on analysis of log-transformed data.

To compare maturity groups for female production, the number of females per petri dish was analyzed in a two-factor design where "trial" was the random factor and "group" was the fixed factor. Only the first two trials were used for CX 366. For CX 366 alone, the numbers of females from the five trials were analyzed as a one-factor design with "trial" as the factor. A two-factor analysis of variance of the data for cultivar and "diapause" was done to compare "diapause" and "nondiapause" months.

RESULTS

Numbers of first-generation *H. glycines* females produced on various soybean cultivars differed significantly ($F = 14.43$; $P < 0.0001$) (Table 1). Cultivars producing the most females in tissue culture were Bass, Williams 82, Kent, Proto, and Chapman. Another five cultivars produced population numbers that were not significantly different from those counted in the highest-producing cultivars, although the numbers were low enough to rate them with less productive cultivars. The differences in population sizes between the highest-ranking and lowest-ranking cultivars were extreme. Lambert and Chesapeake, the cultivars that produced the fewest nematodes, had only 16% as many nematodes as the highest-ranking cultivar, Bass.

Soybean maturity group was correlated with numbers of females produced in root

explant culture (Table 3). Treatments differed significantly ($F = 8.17$, $P < 0.0001$). Maturity groups 1, 2, 6, and 7 had significantly higher female populations than maturity groups 0, 4, 5, and 8. However, even though trends were measured, they could not be used to predict performance of an individual cultivar within a maturity group. For example, in maturity group 3, Bass and Williams produced more nematodes than any other cultivar, SS 390 and CX 366 were intermediate, while Pioneer 9392 was one of the cultivars that produced the fewest females (Table 1). Most of the cultivars in maturity group 4 produced fewer females, but Kent produced large numbers of females. Maturity group 0, which ranked low overall in female population numbers, had one cultivar (Proto) that produced high female populations, while the other cultivar (Lambert) was in the lowest-producing group.

CX 366 was selected for tests to determine whether nematode populations would increase after repeated culturing on a specific soybean cultivar. In the first two trials, CX 366 produced fewer nematode females than the highest-ranking cultivars (including Kent), but the population numbers were not at the bottom of the range (Table 1). Trials 3 and 4 with CX 366 were conducted after five generations in root explant culture on this cultivar, and trial 5 after six generations. Reproduction of this race 3 isolate did not increase on CX 366 after six generations in root explant culture. Mean female numbers per petri dish (reported as least squares means) were as follows: Trial 1, 72.3; Trial 2, 77.3; Trial 3, 107.4; Trial 4, 54.3; Trial 5, 74.2. Trial differences were not significant ($F = 1.54$, $P = 0.2097$).

In the "diapause" analysis, there was a significant influence of cultivar ($F = 4.6$; $P = 0.0331$), of diapause ($F = 12.69$; $P < 0.0001$), and of the cultivar × diapause interaction ($F = 6.65$; $P < 0.0001$). Those cultivars that ranked lowest overall in female numbers tended to produce the fewest females during both the "diapause" and "nondiapause" periods. The cultivars that ranked among the top 10 in nematode populations tended to rank the highest in both the "diapause" months and the "nondiapause" months (Table 2). The exception was Braxton, which ranked high when both trials were combined but had significantly smaller nematode populations during the "diapause" time period. Additionally, SS 390 dropped from midrange overall in the ranking to small numbers of females during the "diapause" months. Braxton, SS 390, and Hutcheson were the only three tested cultivars that demonstrated a decrease in female numbers during the "diapause" time period. More nematodes were produced on Morgan during the "diapause" period than during the "nondiapause" time.

Root dry weights ranged from 0.01 g (cultivars Chapman, Essex, and Braxton) to 0.03 g (cultivars Kent, Duocrop, Hutcheson, Bass, Williams, Pioneer 9392, and Lambert). Based on the estimated standard deviation (0.29), a total sample size of 12 petri dishes is needed to detect a difference of 60 between mean numbers of females per petri dish at the 0.05 significance level.

Discussion

The *H. glycines* race 3 isolate used in this study reproduced well on Kent, on which it had been maintained for some years, and on other soybean cultivars as well. Differences in the mean numbers of females produced on different soybean cultivars demonstrate the value of conducting tests of this nature. The resources invested in root explant cultures produce maximum returns when large numbers of nematodes are harvested, and it is apparent that *H. glycines* populations in monoxenic culture can be more than quadrupled by appropriate cultivar selection. This corroborates earlier work with *Radopholus* spp. on soybean, in which populations produced in root explant cultures varied with soybean cultivar (Huettel, 1989). Numbers of females produced in culture decreased on several soybean cultivars during October through January. It is not known what causes this change in *H. glycines* reproduction in growth chambers, or whether it is correlated with the diapause observed in field studies, so it cannot be ascertained why

the phenomenon occurred on only some soybean cultivars in our tests. However, the results indicate that nematode reproduction does decrease on some cultivars at certain times of year, so nematode production could be increased by use of cultivars that do not demonstrate this effect. Additionally, the data indicated that as few as 12 petri dishes (rather than 20) need to be screened to detect differences in nematode numbers similar to those observed here.

Female population numbers did not appear to be related to the ability of roots to grow in monoxenic culture. Soybean cultivars producing large numbers of nematodes were found at both the top and bottom of the root weight range (i.e. 0.01–0.03 g per root), while cultivars with the lowest nematode populations tended to have roots weighing 0.02 g or more.

There was some difference between the results of this study and those of earlier research (Lauritis et al., 1982) comparing soybean root explant cultures for nematode production. In the previous investigation, female numbers were counted from the 10 susceptible cultivars Essex, Dare, Kent, Lee, Perry, Delmar, Hawkeye, Hood, Hill, and Williams. Only Essex produced significantly more females than the other cultivars (Lauritis et al., 1982), whereas, in our study, nematodes reproduced significantly better on Kent than on Essex. This discrepancy could have been caused by a number of factors: (i) the populations in the two studies were from different geographic locations; (ii) root explant cultures were grown for only 3 weeks in the earlier investigation, allowing less time than in the current experiment for first-generation females to develop; (iii) the earlier study used a different medium for root explant cultures; (iv) the earlier results may not have shown many differences among cultivars because females from only three petri dishes were counted for each cultivar; (v) monoxenic culture on Kent for some years may have increased the ability of this isolate to reproduce on Kent. Research conducted on plants in soil has indicated that repeated culture of soybean cyst nematode on a soybean cultivar increases the nematode's productivity on that cultivar (Young, 1994). In our study, six generations of continuous culture on CX 366 did not increase the numbers of females produced on that cultivar. Either selection for increased productivity is less likely to occur in root explant cultures, or it takes more than six generations to be manifested. Experiments with additional cultivars and increased numbers of generations on each cultivar would have to be conducted to determine the answer to this question.

While there was some correlation between soybean maturity group and number of females produced, maturity group cannot be used to predict which cultivar will provide the most soybean cyst nematodes in root explant cultures. This study indicates that simple procedures, such as selection of individual soybean cultivars, can be used to enhance nematode production for research purposes.

Literature Cited

Fassuliotis, G., and D. P. Bhatt. 1982. Potential of tissue culture for breeding root-knot nematode resistance into vegetables. Journal of Nematology 14:10–14.

Hashmi, G., S. Hashmi, L. R. Krusberg, and R. N. Huettel. 1993. Resistance in *Zea mays* to *Heterodera zeae*. Supplement to the Journal of Nematology 25:820–823.

Hashmi, G., R. N. Huettel, F. A. Hammerschlag, and L. R. Krusberg. 1994. Optimal levels of *Meloidogyne incognita* inoculum for infection of tomato and peach in vitro. Journal of Nematology 26:531–534.

Huettel, R. N. 1989. In vitro screening of soybean lines to *Radopholus similis* and *R. citrophilus*. Nematropica 19:143–149.

Huettel, R. N., and F. A. Hammerschlag. 1986. Influence of cytokinin on in vitro screening of peaches for resistance to nematodes. Plant Disease 70:1141–1144.

Huettel, R. N., and F. A. Hammerschlag. 1993. Response of peach scion cultivars and rootstocks to *Meloidogyne incognita* in vitro and in microplots. Journal of Nematology 25:472–475.

Johnson, R. N., and D. R. Viglierchio. 1969a. Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. I. Selected environmental factors affecting penetration. Nematologica 15:129–143.

Johnson, R. N., and D. R. Viglierchio. 1969b. Sugar beet nematode (*Heterodera schachtii*) reared on axenic *Beta vulgaris* root explants. II. Selected environmental and nutritional factors affecting development and sex ratio. Nematologica 15:144–152.

Ko, M. P., D. P. Schmitt, and B. S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using pluronic F127, including its effects on

population dynamics of *Pratylenchus penetrans*. Journal of Nematology 28:115–123.

Kumar, A., and J. M. S. Forrest. 1990. Reproduction of *Globodera rostochiensis* on transformed roots of *Solanum tuberosum* cv. Desiree. Journal of Nematology 22: 395–398.

Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1982. Screening soybean for resistance to *Heterodera glycines* Ichinohe using monoxenic cultures. Journal of Nematology 14:593–594.

Meyer, S. L. F., and R. N. Huettel. 1996. Application of a sex pheromone, pheromone analogs, and *Verticillium lecanii* for management of *Heterodera glycines*. Journal of Nematology 28:36–42.

Meyer, S. L. F., R. N. Huettel, and R. M. Sayre. 1990. Isolation of fungi from *Heterodera glycines* and in vitro bioassays for their antagonism to eggs. Journal of Nematology 22:532–537.

Meyer, S. L. F., and R. J. Meyer. 1995. Effects of a mutant strain and a wild type strain of *Verticillium lecanii* on *Heterodera glycines* populations in the greenhouse. Journal of Nematology 27:409–417.

Orion, D., W. P. Wergin, and D. J. Chitwood. 1995. Root cortical cell spherical bodies associated with an induced resistance reaction in monoxenic cultures of *Meloidogyne incognita*. Journal of Nematology 27:320–327.

Orion, D., W. P. Wergin, and B. Y. Endo. 1980. Inhibition of syncytia formation and root-knot nematode development on cultures of excised tomato roots. Journal of Nematology 12:196–203.

Rebois, R. V., and R. N. Huettel. 1986. Population dynamics, root penetration, and feeding behavior of *Pratylenchus agilis* in monoxenic root cultures of corn, tomato, and soybean. Journal of Nematology 18:392–397.

Rivera-Smith, C. E., H. Ferris, and R. E. Voss. 1991. The application of an excised root assay for the determination of susceptibility or resistance to root-knot nematodes (*Meloidogyne* spp. Goeldi) in potatoes (*Solanum* spp. L.). American Potato Journal 68:133–142.

Schmitt, D. P. 1992. Population dynamics. Pp. 51–59 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of the soybean cyst nematode. St. Paul, Minnesota: APS Press.

Sudirman, and J. M. Webster. 1995. Effect of ammonium ions on egg hatching and second-stage juveniles of *Meloidogyne incognita* in axenic tomato root culture. Journal of Nematology 27:346–352.

Verdejo, S., and B. A. Jaffee. 1988. Reproduction of *Pasteuria penetrans* in a tissue-culture system containing *Meloidogyne javanica* and *Agrobacterium rhizogenes*-transformed roots. Phytopathology 78:1284–1286.

Yen, J. H., T. L. Niblack, and W. J. Wiebold. 1995. Dormancy of *Heterodera glycines* in Missouri. Journal of Nematology 27:153–163.

Young, L. D. 1994. Changes in reproduction of a *Heterodera glycines* race 5 isolate cultured on "Cordell" and "Bedford" soybean. Supplement to the Journal of Nematology 26:653–655.