

Greenhouse Evaluation of Selected Soybean Germplasm for Resistance to North Carolina Populations of *Heterodera glycines*, *Rotylenchulus reniformis*, and *Meloidogyne* Species¹

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Abstract: Selected soybean genotypes were evaluated for resistance to North Carolina populations of the soybean cyst nematode *Heterodera glycines*, the root-knot nematodes *Meloidogyne incognita* races 3 and 4, *M. arenaria* races 1 and 2, *M. javanica*, and the reniform nematode *Rotylenchulus reniformis* in two greenhouse tests. Populations of cyst nematode used in the first test were cultures from field samples originally classified as races 1-5, and those used in the second test included inbred cyst lines that corresponded to races 1, 3, and 4. The original race classification of some cyst populations shifted after repeated culture on susceptible 'Lee 68' soybean. Most of the cyst-resistant soybean cultivars tested were susceptible to *M. arenaria* and *M. javanica*. Exceptionally large galls were induced by *M. arenaria* on roots of Asgrow 5979, Hartwig, and CNS soybean. Hartwig soybean and PI 437654 were resistant to all cultured field populations of cyst nematodes in a first greenhouse test. In the second test, cyst indices of 11.3% and 19.4% were observed on roots of PI 437654 and Hartwig, respectively, when infected with an inbred line (OP50) of *H. glycines* corresponding to race 4. The cyst-resistant soybean germplasm tested, including Hartwig and PI 437654, supported only low numbers of reniform nematodes. The most severe soybean root necrosis observed, however, was associated with reniform nematode infection.

Key words: *Glycine max*, *Heterodera glycines*, *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, reniform nematode, resistance, root-knot nematode, *Rotylenchulus reniformis*, soybean, soybean cyst nematode.

Plant-parasitic nematodes represent a significant and continuous threat to profitable soybean production in North Carolina and throughout the United States (5,19). In 1991, North Carolina soybean yield losses due to damage by nematodes were estimated at 8.5%, with calculated losses of approximately \$19 million to soybean growers in the state (19). Crop rotation and the use of nematode-resistant soybean cultivars remain the most effective nematode management tactics available to soybean growers (8). The major pathogen of soybean in North Carolina and the United States is the soybean cyst nematode, *Heterodera glycines* (10). Resistance to races 1 and 3, derived from Peking, and

racess 9 and 14, derived from PI 88788, are available in agronomically acceptable soybean cultivars grown in the southeast. Potential resistance to race 2, derived primarily from PI 90763, is important since race 2 is found in relatively high frequency in North Carolina when compared to other states (8). Resistant soybean cultivars provide cost-effective management, but their utility often is diminished upon continuous cropping due to selection for increases in populations of the nematode that are able to parasitize resistant cultivars (13). The recently released cultivar, Hartwig, was developed from a cross between PI 437654 and Forrest and currently is resistant to all races of soybean cyst nematode tested (2). Hartwig is used in both public and private soybean breeding programs to develop agronomically superior soybean cultivars with cyst nematode resistance.

A problem associated with cultivation of cyst-resistant soybean cultivars in North Carolina is the potential damage caused by other nematode species, particularly root-knot (*Meloidogyne* species) and reniform (*Rotylenchulus reniformis*) nematodes (8). *Meloidogyne incognita*, *M. arenaria*, and *M.*

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javanica are the species of root-knot nematodes important in North Carolina soybean crops. The occurrence of mixed species and races of plant-parasitic nematodes within a soybean field often occurs in North Carolina and limits the usefulness of available nematode-resistant soybean cultivars. Soybean genotypes grown in the southern United States have been evaluated for resistance to cyst, root-knot, and reniform nematodes in several studies (7, 14–17). Recently, late-maturing soybean cultivars bred for high levels of resistance to cyst and root-knot nematodes have been released (3). The research reported here was designed to evaluate resistance in selected soybean genotypes to multiple populations of cyst, root-knot, and reniform nematodes present in North Carolina.

MATERIALS AND METHODS

Several soybean cultivars bred for high yield in the southeastern United States and

selected soybean germplasm developed primarily for resistance to the soybean cyst nematode were evaluated for resistance to North Carolina populations of soybean cyst, root-knot, and reniform nematodes in two greenhouse tests. The soybean cultivars and germplasm evaluated for resistance against the nematode populations are listed in Tables 1–4. The nematode inocula used in greenhouse Test 1 included (i) field populations of soybean cyst nematode, classified as races 1–5 when collected from North Carolina soybean growing regions, and subsequently cultured on susceptible 'Lee 68' soybean in the greenhouse; (ii) populations of races 3 and 4 of *Meloidogyne incognita*, races 1 and 2 of *M. arenaria*, and a population of *M. javanica* that were isolated from North Carolina field samples and maintained in pure greenhouse culture on roots of 'Rutgers' tomato (*Lycopersicon esculentum*); (iii) one field population of reniform nematode

TABLE 1. Gall indices^a and egg production of greenhouse-cultured^b North Carolina populations of *Meloidogyne incognita* races 3 and 4, *M. arenaria* races 1 and 2, and *M. javanica* on selected soybean cultivars in two greenhouse tests.

| Soybean | <i>M. incognita</i> race 3 | | <i>M. incognita</i> race 4 | | <i>M. arenaria</i> race 1 | | <i>M. arenaria</i> race 2 | | <i>M. javanica</i> | |
|---------------|----------------------------|-------------------------------|----------------------------|-------------------------------|---------------------------|-------------------------------|---------------------------|-------------------------------|--------------------|-------------------------------|
| | GI | Eggs/ root sys. (×1000) | GI | Eggs/ root sys. (×1000) | GI | Eggs/ root sys. (×1000) | GI | Eggs/ root sys. (×1000) | GI | Eggs/ root sys. (×1000) |
| Test 1 | | | | | | | | | | |
| Asgrow 5979 | 44 b | 46.2 a | 41 a | 41.4 a | 55 ab | 90.5 a | 58 bc | 240.5 a | 52 ab | 188.6 a |
| CNS | 38 b | 104.4 a | 29 b | 29.7 a | 61 a | 92.8 a | 78 a | 237.6 a | 68 a | 147.1 a |
| Centennial | 1 c | 1.2 c | 2 c | 2.9 ab | 65 a | 51.5 a | 67 ab | 62.9 ab | 56 ab | 25.3 a |
| Lee 68 | 68 a | 70.5 a | 45 a | 15.1 a | 44 bc | 29.5 a | 44 cd | 42.5 ab | 22 c | 3.5 b |
| NK S61-89 | 1 c | 3.5 bc | 1 c | 0.3 c | 33 cd | 13.4 b | 33 de | 39.9 ab | 4 d | 1.1 b |
| Hartwig | 10 c | 1.7 c | 1 c | 1.0 bc | 60 a | 17.3 b | 69 ab | 49.6 b | 49 b | 32.7 a |
| Jackson | 6 c | 21.7 ab | 6 c | 6.8 a | 29 cd | 13.5 ab | 36 de | 51.6 ab | 10 cd | 1.4 b |
| Bryan | 0 c | 2.3 bc | 3 c | 0.7 bc | 23 d | 4.5 b | 25 e | 22.4 b | 4 d | 0.7 b |
| Test 2 | | | | | | | | | | |
| Asgrow 5979 | 8 bc | 26.1 b | 15 b | 18.3 b | 43 a | 199.8 a | 37 a | 152.0 a | 43 a | 225.6 a |
| CNS | 15 a | 73.5 a | 15 b | 74.9 a | 38 ab | 103.8 b | 42 a | 122.6 a | 33 b | 148.2 ab |
| Centennial | 7 bc | 4.9 b | 10 bc | 7.9 b | 22 c | 43.7 bc | 25 b | 59.6 b | 22 c | 81.4 bc |
| Lee 68 | 12 ab | 11.3 b | 23 a | 72.0 a | 23 c | 25.9 c | 22 bc | 60.8 b | 8 d | 6.3 c |
| NK S61-89 | 5 c | 2.1 b | 7 c | 4.6 b | 15 c | 32.3 bc | 7 bc | 19.1 b | 8 d | 8.4 c |
| Hartwig | 5 c | 4.5 b | 8 c | 3.1 b | 22 c | 36.9 bc | 40 a | 138.4 a | 32 b | 90.7 bc |
| Jackson | 8 bc | 8.2 b | 8 c | 12.1 b | 27 bc | 204.0 a | 18 bc | 45.8 b | 13 cd | 13.8 c |
| Bryan | 5 c | 5.0 b | 5 c | 1.9 b | 17 c | 52.6 bc | 12 c | 12.8 b | 12 d | 4.7 c |

Means followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t* test, *k* = 50). Table values for Test 1 and Test 2 are the mean of four and three replicate treatments, respectively.

^a Table values of gall indices (GI) represent the percentage of total root system galled, and egg production is represented as total eggs extracted per root system × 1000 (eggs/root system).

^b Field isolates of root-knot nematodes from North Carolina were identified and maintained in pure culture on roots of 'Rutgers' tomato in the greenhouse for use as inoculum in this experiment. Initial inoculum was 10,000 eggs/root system in both tests.

TABLE 2. Number of cysts^a produced by greenhouse-cultured North Carolina field populations of races 1–5 of *Heterodera glycines* on selected soybean genotypes in greenhouse Test 1.

| Soybean | Race 5 (1) ^b | | | Race 2 | | | Race 1 (3) | | | Race 2 (4) | | | Race 2 (5) | | |
|--------------|-------------------------|-------|---------------------|--------|-------|--------|------------|-------|--------|------------|-------|--------|------------|-------|--------|
| | cysts | Index | Rating ^c | cysts | Index | Rating | cysts | Index | Rating | cysts | Index | Rating | cysts | Index | Rating |
| Lee 74 | 564 a | 100.0 | S | 753 a | 100.0 | S | 339 a | 100.0 | S | 598 b | 100.0 | S | 531 a | 100.0 | S |
| Pickett | 57 d | 10.0 | MR | 407 c | 54.1 | MS | 6 d | 1.6 | R | 516 bc | 86.2 | S | 441 a | 83.0 | S |
| Peking | 10 d | 1.8 | R | 494 bc | 65.6 | S | 9 d | 2.5 | R | 262 de | 43.8 | MS | 235 bc | 44.2 | MS |
| PI 88788 | 81 d | 14.4 | MR | 314 c | 41.7 | MS | 122 c | 35.9 | MS | 393 cd | 65.7 | S | 219 bc | 41.2 | MS |
| PI 90763 | 20 d | 3.5 | R | 33 d | 4.4 | R | 21 d | 6.1 | R | 19 fg | 3.2 | R | 2 d | 0.4 | R |
| Pioneer 9521 | 23 d | 4.1 | R | 828 a | 110.0 | S | 1 d | 0.3 | R | 827 a | 138.3 | S | 487 a | 91.7 | S |
| TN5-92 | 2 d | 0.3 | R | 488 bc | 64.8 | S | 65 cd | 19.1 | MR | 160 efg | 26.7 | MR | 145 cd | 27.3 | MR |
| Asgrow 5979 | 369 b | 65.4 | S | 329 c | 43.7 | MS | 201 b | 59.4 | MS | 411 cd | 68.7 | S | 549 a | 103.4 | S |
| NK S61-89 | 386 bc | 68.4 | S | 352 c | 46.8 | MS | 203 b | 60.0 | MS | 761 a | 127.2 | S | 358 ab | 67.5 | S |
| TCPR 90-172 | 301 c | 54.2 | MS | 696 ab | 92.4 | S | 267 ab | 78.7 | S | 407 cd | 68.0 | S | 215 bc | 40.5 | MS |
| Cordell | 2 d | 0.4 | R | 380 c | 50.5 | MS | 1 d | 0.1 | R | 166 cf | 27.8 | MR | 82 cd | 15.4 | MR |
| Hartwig | 5 d | 0.9 | R | 1 d | 0.1 | R | 0 d | 0.0 | R | 2 g | 0.3 | R | 4 d | 0.7 | R |
| PI 437654 | 4 d | 0.6 | R | 4 d | 0.5 | R | 19 d | 5.6 | R | 2 g | 0.3 | R | 4 d | 0.7 | R |

Means followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t* test, *k* = 50). Table values represent the mean of five replicate treatments.

^a Number of cysts per root system (cysts) and cyst indices (Index) calculated as the percentage number of cysts per root system as compared to susceptible 'Lee 68' soybean.

^b Field isolates of SCN were cultured on roots of susceptible 'Lee 68' soybean in the greenhouse for use as inoculum in this experiment. Initial inoculum was 10,000 eggs per root system. Race designations at end of test are listed, and the original race classifications for field isolates upon collection are represented in parentheses.

^c Rating is based upon scale presented by Schmitt and Shannon (18), where cyst index 0–9 is resistant (R), cyst index 10–30 is moderately resistant (MR), cyst index 31–60 is moderately susceptible (MS), and cyst index >60 is susceptible (S).

TABLE 3. Number of cysts^a produced by inbred lines^b ("races") of *Heterodera glycines*, and a greenhouse-cultured^c North Carolina field population of race 2 of *H. glycines*, on selected soybean genotypes in greenhouse Test 2.

| Soybean | Inbred "Race 1" | | | Race 4 (2) ^c | | | Inbred "Race 3" | | | Inbred "Race 4" | | |
|--------------|-----------------|-------|---------------------|-------------------------|-------|--------|-----------------|-------|--------|-----------------|-------|--------|
| | Cysts | Index | Rating ^d | Cysts | Index | Rating | Cysts | Index | Rating | Cysts | Index | Rating |
| Lee 74 | 782 a | 100.0 | S | 603 a | 100.0 | S | 259 a | 100.0 | S | 542 ab | 100.0 | S |
| Pickett | 1 d | 0.0 | R | 285 bc | 47.3 | MS | 25 bc | 9.7 | R | 186 c | 34.3 | MR |
| Peking | 5 d | 0.6 | R | 89 cde | 14.8 | MR | 24 bc | 9.3 | R | 164 c | 30.3 | MR |
| PI 88788 | 393 bc | 50.3 | MR | 85 cde | 14.1 | MR | 33 bc | 12.7 | MR | 135 c | 24.9 | MR |
| PI 90763 | 1 d | 0.1 | R | 336 b | 55.7 | MS | 10 bc | 3.5 | R | 604 a | 111.4 | S |
| Pioneer 9521 | 4 d | 0.5 | R | 257 bcd | 42.6 | MS | 140 abc | 54.1 | (0.3) | 312 bc | 57.6 | MS |
| TN5-92 | 112 cd | 14.3 | MR | 69 de | 11.4 | MR | 12 bc | 4.6 | (19.1) | 607 a | 112.0 | S |
| Asgrow 5979 | 476 b | 60.9 | S | 79 cde | 13.1 | MR | 10 bc | 3.9 | (59.4) | 133 c | 24.5 | MR |
| NK S61-89 | 921 a | 117.8 | S | 108 cde | 17.9 | MR | 62 bc | 23.9 | MR | 162 c | 29.9 | MR |
| TCPR 90-172 | 472 b | 60.4 | S | 151 bcde | 25.0 | MR | 153 ab | 59.1 | MS | 195 c | 36.0 | MS |
| Cordell | 159 cd | 20.3 | (0.4) | 197 bcde | 32.7 | MS | 11 bc | 4.2 | R | 633 a | 116.8 | S |
| Hartwig | 31 d | 4.0 | R | 5 e | 0.8 | R | 2 c | 0.7 | R | 105 c | 19.4 | (0.3) |
| PI 437654 | 5 d | 0.6 | R | 68 de | 11.3 | (0.5) | 1 c | 6.6 | R | 61 c | 11.3 | (0.3) |

^a Means followed by the same letter are not significantly different (Waller-Duncan *k*-ratio *t* test, *k* = 50). Table values represent the mean of four replicate treatments.

^b Number of cysts per root system (cysts) and cyst indices (Index) calculated as the percentage number of cysts per root system as compared to susceptible 'Lee 68' soybean.

^c "Race 1" (OP20), "Race 3" (OP25), and "Race 4" (OP50) are inbred lines developed from crosses of single SCN individuals and subsequent sib matings (11). Inbred "Race 3" may contain contaminating SCN that now classifies this population as race 1.

^d This population of SCN is now classified as race 4. It was originally classified as race 2 (parentheses) when collected from the field and was subsequently cultured on roots of susceptible 'Lee 68' soybean in the greenhouse for use as inoculum in this experiment. Initial inoculum was 10,000 eggs per root system for all nematode cultures used in Test 2.

^e Rating is based upon scale presented by Schmitt and Shannon (18), where cyst index 0–9 is resistant (R), cyst index 10–30 is moderately resistant (MR), cyst index 31–60 is moderately susceptible (MS), and cyst index >60 is susceptible (S). Cyst index values in parentheses differ substantially and are from the same soybean genotypes tested against the corresponding field races of SCN in Test 1 (Table 2).

TABLE 4. Final populations^a of greenhouse-cultured^b North Carolina (NC) and Georgia (GA) populations of *Rotylenchulus reniformis* grown on selected soybean cultivars in two greenhouse tests^c.

| Soybean | Test 1 No. eggs and vermiforms | | Test 2 No. eggs and vermiforms |
|--------------|--------------------------------------|-----------|--------------------------------------|
| | NC | GA | NC |
| Braxton | 15,862 a | 13,410 ab | 39,952 a |
| Asgrow 5979 | 14,875 a | 10,082 b | 26,530 ab |
| NK S61-89 | 5,479 ab | 11,670 b | 12,586 b |
| Forrest | 1,476 bc | 1,212 cd | 326 c |
| Pioneer 9521 | 775 cd | 1,041 cd | 789 c |
| Hartwig | 800 cd | 2,785 c | 406 c |
| TN5-92 | 462 d | 1,253 cd | 198 c |
| PI 437654 | 853 cd | 490 d | 344 c |

Means followed by the same letter are not significantly different (Waller-Duncan *k*-ratio, *t* test, *k* = 50). Table values represent the mean of four replicate treatments.

^a Reproduction was measured as the total number of reniform nematode eggs and mixed vermiform life stages (no. eggs and vermiforms) extracted from a 500-cm³ soil sample from 15-cm-diam. pots in Test 1 and from the entire 15-cm-diam. pot of soil in Test 2.

^b Field isolates of reniform nematode were cultured on roots of 'Deltapine' cotton in the greenhouse for use as inoculum in this experiment. Initial inoculum was 10,000 eggs and vermiforms/pot in Test 1, and 8,000 eggs and vermiforms/pot in Test 2.

^c The GA population of reniform nematode was not available for Test 2.

from soybean grown in North Carolina and another reniform population from Georgia that were maintained in greenhouse culture on roots of 'Deltapine 16' cotton (*Gossypium hirsutum*). In Test 2, the same root-knot nematode populations and the reniform population from North Carolina (only) were used as inocula; however, race 5 of soybean cyst nematode was omitted and the cultured field populations of races 1, 3, and 4 were replaced with the highly homozygous, soybean cyst nematode inbred lines OP20, OP25, and OP50 (11) that correspond, respectively, to these field races. The term "inbred races" is used here for convenience, but these cyst nematode lines actually do not represent "races" since they are derived from individual nematode crosses and sibling matings (11).

All soybean lines were tested against nematodes in 15-cm-diam. clay pots containing a 1:1 sand to silty loam soil mixture. In Test 1, soybean seeds were germinated in Metromix 220 (Scotts-Sierra,

Marysville, OH) for 3 days, and the seedlings were then transplanted to the pots. All nematode inoculum was introduced into the soil immediately below the seedlings upon transplanting in Test 1. In Test 2, soybean seed was germinated directly in the experimental pots containing the sand-soil mix, and nematode inoculum was added to the soil at the base of each plant 2 weeks after emergence. Cysts of *H. glycines* were collected from roots and soil of greenhouse cultures using spray from a high-water-pressure hose combined with floating and sieving, and then cysts were crushed with a Ten-Brock homogenizer to release eggs for use as inoculum (7). The soil in each pot was infested with 10,000 eggs in both tests, and after 50 days plants were harvested and total cysts were extracted from soil and roots of individual plants by flotation and sieving. A visual assessment of the percentage of total root system necrosis also was recorded at harvest, as well as the fresh weight of each root system. Each treatment (soybean cultivar plus cyst nematode population) was replicated five times in Test 1 and four times in Test 2.

Eggs of root-knot nematode populations were extracted from roots of greenhouse cultures using NaOCl (6), and 10,000 eggs were used to infest each pot in both Tests 1 and 2. Each treatment (cultivar plus root-knot nematode population) was replicated four times in Test 1 and three times in Test 2. Plants were harvested at 56 days after inoculation and rated visually for the percentage of root system galled and percentage root system exhibiting necrosis. Root fresh weights were recorded concomitantly. Eggs of root-knot nematode from infected soybean roots were extracted from a random 5-g sample of each root system with NaOCl (6) and used to calculate total eggs per root system based upon total root fresh weight.

A mixture of both egg and vermiform life stages of the reniform nematode was extracted by flotation and sieving from greenhouse cultures and used as inoculum for soybean tests. In Test 1, each soybean

plant was inoculated with a total of 10,000 reniform eggs and juveniles; in Test 2, plants were inoculated with a total of 8,000 eggs and juveniles. Each treatment (soybean cultivar plus reniform nematode population) was replicated four times. Soybean was harvested at 90 days after inoculation in both tests. At harvest in Test 1, eggs and vermiform stages of reniform nematode were extracted from a 500-cm³ soil sample by semi-automatic elutriation (4), whereas in Test 2, eggs and juveniles were extracted by sieving and flotation of all soil in individual pots (7). The percentage root system showing necrosis and total fresh root weight were recorded at harvest of reniform nematode-inoculated soybean plants.

Greenhouse tests were arranged in a randomized complete block design with cultivar and nematode population as the main treatments. Nematode data were transformed by $\log_{10}(x + 1)$ to standardize the variance, but untransformed data are presented in tables for clarity. All data were subjected to analysis of variance using the SAS General Linear Models procedure, and interaction main effects of Test 1 and Test 2 were analyzed for all dependent variables presented above. The Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 50) was used to compare treatment means.

RESULTS

Analysis of variance for each test indicated that cultivar effects were significant ($P \leq 0.05$) for all nematode species. Significant interactions for the dependent variables presented in Tables 1–4 occurred between Test 1 and Test 2; hence, values for both tests are listed separately. Resistance to several populations of root-knot nematodes differed among the selected soybean cultivars, and the results of the second test generally confirmed the results of the first test (Table 1). Overall, 'Bryan' soybean demonstrated a consistently high level of resistance to root-knot nematodes with the exception of *M. arenaria*. Several of the soybean cyst nematode-resistant and high-

yielding soybean cultivars were susceptible to root-knot nematodes, especially *M. arenaria*. Gall ratings for both races of *M. arenaria* were relatively high in both tests on most cultivars tested, especially for 'Asgrow 5979', 'CNS', 'Hartwig' soybean. 'Asgrow 5979', 'CNS', and 'Lee 68' soybean were susceptible to all root-knot nematode populations tested, except for some moderate resistance of Lee 68 to *M. javanica*. 'Centennial', 'Northrup King S61-89', and 'Hartwig' soybean had high levels of resistance to *M. incognita* races 3 and 4, and NK S61-89 was moderately resistant to *M. javanica* as well. 'Jackson' soybean had moderate resistance to *M. incognita* races 3 and 4 and *M. javanica* in both greenhouse tests.

In both tests of soybean germplasm for resistance to the soybean cyst nematode, the soybean host race differentials (Lee, Pickett, Peking, PI 88788, and PI 90763) and PI 437654, the source of resistance in Hartwig soybean, were included with the selected soybean cultivars. Reproduction of the soybean cyst nematode populations on host differentials indicated that the original field populations of races 1, 3, 4, and 5 that were used in Test 1 had shifted to races 5, 1, 2, and 2, respectively, in greenhouse culture on susceptible Lee 68 soybean. In Test 2, the cultured field population of SCN race 2 had become race 4, and the inbred race 3 had become race 1. The results (cyst indices) of Test 1 (Table 2) were generally confirmed by the results of Test 2, except where noted in parentheses in Table 3. The scale of soybean responses adapted by Schmitt and Shannon (18) is used in Tables 2 and 3 to classify the range of susceptible and resistant responses to soybean cyst nematodes observed herein. NK S61-89 and TCPR 90-172 soybean were susceptible to all soybean cyst populations in both tests. Asgrow 5979 was susceptible to all cyst races in Test 1 and was resistant to the inbred race 3 population (now race 1) in Test 2. Pioneer 9521 was resistant to races 5 (originally race 1) and 1 (originally race 3) of soybean cyst nematode in Test 1, but resistant to only the inbred race 1 population in

Test 2. 'Cordell' also was resistant to races 5 (originally race 1) and 1 (originally race 3) in Test 1, and resistant to only the inbred race 3 population in Test 2. TN5-92 was resistant to only race 5 (originally race 1) in Test 1, and resistant to only the inbred line of race 3 in Test 2. Hartwig and PI 437654 were highly resistant to all cyst populations used in Test 1, but PI 437654 was susceptible to field race 4 (originally race 2) and inbred race 4 in Test 2, and Hartwig also was susceptible to inbred race 4 in Test 2.

The number of eggs and vermiform life stages of the reniform nematode isolated from inoculated soybean cultivars followed the same pattern in Tests 1 and 2 (Table 4). The final population densities of the North Carolina and Georgia populations of reniform nematode were comparable on each of the soybean cultivars evaluated in Test 1. 'Braxton', NK S61-89, and Asgrow 5979 soybean supported relatively high population densities of reniform nematode, although the number of eggs and vermiform stages recovered from NK S61-89 were relatively moderate compared to the other two cultivars. Forrest, Pioneer 9521, TN5-92, Hartwig, and PI 437654 were relatively resistant to reniform nematode.

Analysis of variance for root system necrosis revealed no differences ($P \leq 0.05$) in at least one of two tests for each of the soybean germplasm-nematode combinations tested (data not shown), yet some significant effects and trends of nematode-associated root necrosis of soybean lines were observed. Root-knot nematodes significantly affected ($P \leq 0.06$) soybean root necrosis in Test 1, especially with *M. arenaria*. Mean root system necroses with *M. arenaria* race 2 were 56%, 36%, and 35% for CNS, Hartwig, and Asgrow 5979, respectively. The least root necroses among all root-knot nematode species tested were observed with NK S61-89 and Bryan soybean. Root necrosis indices among the soybean cultivars inoculated with reniform nematodes did not differ significantly ($P \leq 0.05$). Overall, however, more extensive

soybean root necrosis was observed with infection by reniform nematodes than with root-knot or cyst nematodes. TN5-92, Asgrow 5979, and NK S61-89 inoculated with reniform nematodes experienced mean root system necrosis of 42%, 35%, and 35%, respectively, whereas Hartwig soybean demonstrated 22% mean root system necrosis. The race 4 field (originally race 2) culture and inbred races 3 (now race 1) and 4 of soybean cyst nematode were associated with enhanced root system necrosis ($P \leq 0.05$) of soybean lines evaluated in Test 2. Pioneer 9521, Asgrow 5979, and Lee 68 soybean inoculated with these cyst populations had the highest percentage of root system necrosis, whereas TCPR 90-172 and PI 437654 exhibited the lowest necrosis ratings.

DISCUSSION

The results of these greenhouse screenings indicate that many of the selected soybean genotypes are susceptible to one or more of the North Carolina populations of root-knot, soybean cyst, or reniform nematodes evaluated. The origin of the nematode population may be critical in evaluating soybean lines for nematode resistance. For example, Asgrow 5979, a soybean cultivar that yields relatively well in the southeastern United States and is reported as being resistant to Arkansas populations of soybean cyst nematode (16), was susceptible to most of the nematode populations tested, including the majority of the cyst nematode populations tested. Most of the soybean genotypes tested, including cyst-resistant cultivars, were moderate to highly resistant to *M. incognita* races 3 and 4, but almost all were susceptible to *M. arenaria* races 1 and 2 and *M. javanica*, except for Bryan and NK S61-89 soybean, which were relatively resistant in most tests. Large galls and considerable root necrosis were associated with *M. arenaria* infection of several cyst-resistant soybean genotypes, including Hartwig. In general, root-gall indices were indicative of root-knot nematode egg production, but the observed ex-

ceptions highlight the value of obtaining both types of data.

Difficulty in maintaining the race identity of soybean cyst nematode field populations in greenhouse culture was apparent from the host differential results obtained here. Factors other than host suitability may have been responsible for the observed shifts in soybean cyst nematode race identity since no host selection should have been applied from the susceptible 'Lee 68' soybean used for greenhouse culture. Other researchers have suggested that edaphic factors and differential reproduction rates may substantially contribute to selecting the proportion of different soybean cyst nematode genotypes that constitute any given population (1,9). Differential final populations of these cultured soybean cyst nematode field races were obtained, however, on several of the soybean genotypes evaluated. Hartwig soybean and PI 437654 were highly resistant, but Asgrow 5979, TCPR 90-172, and NK S61-89 were highly susceptible to the cultured field populations of races 1-5 tested here. Inbred lines of soybean cyst nematodes (11) were substituted for field cultures in Test 2 to circumvent observed "race shifts," but one of these inbred lines (OP25) corresponding to race 3 was most likely lightly contaminated since it was subsequently classified as a marginal race 1 on differential soybean hosts. In general, final populations of the different soybean cyst nematode cultures were comparable on the selected soybean genotypes in both tests with two notable exceptions. Pioneer 9521 supported substantially higher populations of inbred race 3 (now race 1) compared to the field culture of race 3 (now race 1), and the converse was true for Asgrow 5979 and TN5-92. Of more considerable interest is the recovery of cysts of the race 2 field culture (now race 4) on PI 437654 (cyst index = 11.3) and the inbred line that corresponds to race 4 (OP50) on both Hartwig (cyst index = 19.4) and PI 437654 (cyst index = 11.3). The progeny of OP50 from Hartwig and PI 437654 were viable (Davis, unpubl.), but it is yet

unknown if they can multiply to substantial numbers on these soybean genotypes. However, these data indicate that the genetic capacity to reproduce on PI 437654 and Hartwig soybean exists within the soybean cyst nematode.

Braxton and Asgrow 5979 were highly susceptible to both the North Carolina and Georgia populations of reniform nematode, which generally agrees with previous reports (17). Soybean genotypes that are resistant to soybean cyst nematode, including Forrest, Hartwig, and PI 437654, were also resistant to reniform nematode in these experiments. As some results of previous reports suggest (12,17), resistance to soybean cyst nematode in soybean may be linked to resistance to reniform nematode. Interestingly, the relative amount of root necrosis associated with reniform nematode infection of soybean in our experiments was considerably greater than that observed with cyst or root-knot nematodes, especially on susceptible soybean genotypes. The results of these experiments indicate that soybean growers and breeders in North Carolina and the southeastern United States must consider multiple populations of nematodes, including soybean cyst, root-knot, and reniform nematodes, when selecting or developing soybean cultivars that are nematode-resistant.

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