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Survey of *Heterodera glycines* Races and Other Plant-parasitic Nematodes on Soybean in North Carolina[1]

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Abstract: A survey of soybean-production areas in the Piedmont, Coastal Plain and Tidewater regions of North Carolina was conducted from 1994 to 1996. *Heterodera glycines* was detected in 55 of 77 fields sampled in 15 counties. The host race of *H. glycines* was determined for 39 of the populations collected. Of all populations collected, 4% were race 1, 40% race 2, 16% race 4, 7% race 5, and 4% race 9; the remaining 29% could not be accurately categorized. None of the populations evaluated had high levels of reproduction on the resistant cultivar Hartwig. The southern root-knot nematode *Meloidogyne incognita* was detected in 26% of the fields. *Helicotylenchus* spp. were detected in all fields sampled, *Tylenchorhynchus* spp. were found in 62%, *Paratrichodorus* spp. in 56%, and *Pratylenchus* spp. in 72% of fields sampled. *Mesocriconema* spp., *Xiphinema* spp., and *Hoplolaimus* spp. were detected in less than 20% of the fields sampled.

Key words: Distribution, *Glycine max, Helicotylenchus, Heterodera glycines, Hoplolaimus columbus, Hoplolaimus galeatus,* host race, *Meloidogyne incognita, Mesocriconema,* nematode, *Paratrichodorus,* populations, *Pratylenchus,* race, soybean, soybean cyst nematode, survey, *Tylenchorhynchus, Xiphinema.*

The soybean cyst nematode, *Heterodera glycines* Ichinohe, was first discovered in the United States in New Hanover County, North Carolina, in 1954 (Noel, 1992; Winstead et al., 1955). This nematode is currently the most serious pathogen of soybean in the Americas (Wrather et al., 1997). Cultural practices and resistant cultivars are the primary means of managing this nematode (Wrather et al., 1992; Young, 1996a, 1996b). The use of resistant cultivars places selection pressure on populations of *H. glycines*, resulting in changes in the frequency of alleles for parasitism (Triantaphyllou, 1975). This phenomenon is referred to as a race shift for advisory purposes, and results in populations of *H. glycines* that can parasitize previously resistant cultivars (Young, 1984; Young et al., 1986). Because of the genetic variability of this pathogen, periodic assessments of the ability of field populations to reproduce on resistant cultivars are required (Niblack et al., 1993; Riggs et al., 1988; Young, 1990).

Schmitt and Barker (1987) found *H. glycines* in 33% of soybean fields in the North Carolina Coastal Plain sampled in 1985 and 1986. Percentages of the populations at that time were categorized as race 1, 18%; race 2, 21%; race 3, 15%; race 4, 7%; race 5, 16%; and others, 23%. These data suggest that resistance to races 1 and 3 derived from Peking would no longer be effective in managing prevalent populations of *H. glycines* in many fields in the state. The cultivar Bedford, with resistance from PI88788 and Peking, was released as resistant to races 3 and 4 of soybean cyst nematode (Hartwig and Epps, 1977). This cultivar was not well accepted in North Carolina (E. J. Dunphy, personal communication), but cultivars with Bedford-type resistance to races 3, 9, and 14 have gained acceptance in recent years. Still, these cultivars have only limited resistance to races 2 and 4, which predominate in North Carolina (Schmitt and Barker, 1987). Cultivars with resistance from sources other than Peking or PI88788 would be useful in managing other races of *H. glycines*, such as host races 2, 4, and 5. The only cultivars with different types of resistance available to southeastern growers are Cordell (Hartwig and Young, 1990), TN5-92 (Davis et al., 1996), Northrup King S61-89 (all with PI90763 in their pedigree), and the cultivar Hartwig with Forrest (Peking background) and PI437654 in its pedigree (Anand, 1992). The cultivar Hartwig is considered resistant to all host races of SCN in the United States

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at this time. Although these cultivars have different resistance to *H. glycines,* they have not been well received by growers because of poor agronomic characteristics. Cordell, TN5-92, and Northrup King S61-89 are no longer available to growers, and Hartwig is to be replaced by Delsoy 5710 (S. C. Anand, personal communication).

Successful management of plant-parasitic nematodes must be directed toward the prevalent species and, where soybean is concerned, the most common races of soybean cyst nematode. Research and extension resources can be best targeted to the major problems if the frequency of occurrence of parasitic nematode species and races are characterized.

The primary objective of this research was to ascertain the frequency of occurrence of races of *H. glycines* in North Carolina, and their ability to reproduce on available, resistant cultivars. A secondary objective included determining the prevalence of other plant-parasitic nematodes on soybean.

Materials and Methods

A survey of 15 soybean-producing counties in the Piedmont, Coastal Plain, and Tidewater regions of North Carolina was conducted each September in 1994, 1995, and 1996 (Table 1). With the aid of cooperative extension personnel, fields were selected from each county as being representative of the various cultural systems (i.e., rotation, cropping sequence, and tillage practices) employed in that county. Sampling area was predetermined to be 2 ha, from which approximately 8 liters of soil was collected. Samples consisted of 2.5-cm-diam. soil cores taken to a depth of 15–20 cm in an X or Z pattern across the area sampled. Cores were composited and mixed for processing.

Nematodes were assayed from two 500-cm^3 soil samples from each field and the results averaged. Samples were processed by elutriation, modified centrifugation, and mist chamber (Barker et al., 1986). A portion of the remaining soil was placed in 6-cm-diam. clay pots in the greenhouse. Seeds of the soybean cyst nematode host race differential cultivars or liens were planted with Essex (substituted for Lee), Pickett, Peking, PI88788, and PI90763 (Riggs and Schmitt, 1988). Additional cultivars were used to identify their relative resistance to field populations of soybean cyst nematode. The supplemental differentials include Northrup King S61-89 (resistant to

TABLE 1. North Carolina counties sampled for nematodes (1994–1996), year sampled, soybean hectares harvested in year sampled, detection of soybean cyst nematode (*Heterodera glycines*), and host races found.

County	Year	Sample numbers	Hectares harvested (Thousands)[a]	Cysts detected	Races detected
Beaufort	1996	58–62	18.4	+	1, 2, 4
Camden	1995	33–37	10.6	+	2, 4, 9
Columbus	1996	73–77	17.6	+	4
Cumberland	1995	48–52	5.6	+	1, 2, 5
Franklin	1996	68–72	8.6	+	—
Hyde	1996	53–57	13.8	+	2
Johnston	1996	63–67	21.4	+	2, 5
Pasquotank	1995	27–32	17.2	+	2
Pitt	1994	22–26	19.4	+	2, 4
Robeson	1994	16–21	42.9	+	2
Sampson	1994	11–15	22.2	+	2, 4
Stanly	1995	38–42	6.4	–	—
Tyrrell	1994	1–5	12.9	+	2, 5
Union	1995	43–47	17.3	–	—
Washington	1994	6–10	16.2	+	2, 4, 5

[a] North Carolina Agricultural Statistics 1994, 1995, 1996.

races 3, 9 and 14), TN5-92 (Cordell-type resistance—races 1, 3, and 5), Hartz 5164 (resistant to races 3 and 14), and Hartwig (resistant to all races). Each differential test was replicated three times. Host differential plants were removed from the soil 4 to 6 weeks after planting, and cysts were washed from roots and counted. Terminology developed by Schmitt and Shannon (1992) was used to discriminate among cultivars and their relative resistance.

In samples where root-knot nematodes were extracted at population densities greater than 80 J2/500 cm^3 soil, a subsample of soil was placed in 17-cm-diam. pots and tomato (*Lycopersicum esculentum* cv. Rutgers) seedlings were transplanted and maintained for 12 weeks. Species of root-knot nematodes were determined by examination of perineal patterns (Hartman and Sasser, 1985). Species of *Hoplolaimus* and *Pratylenchus* were determined through microscopic examination of semi-permanent mounts, provided sufficient adults were present in samples to provide for accurate identification.

Records of soybean cyst nematode infestation of North Carolina counties have been maintained since 1954. Since 1975, reports of soybean cyst nematode in previously uninfested counties have been verified by site visits. Soil samples were collected, and the races of this nematode were determined (Golden et al., 1970). Information on infested counties is presented in Figure 1.

Results

Soybean cyst nematode was detected in 13 of the 15 counties sampled. Cysts of *H. glycines* were found in 71% of the fields sampled, whereas eggs and juveniles were detected in 68% and 60% of the fields, respectively.

Populations characterized as to host race were race 1, 4%; race 2, 40%; race 4, 17%; race 5, 4%; and race 9, 4%; 29% could not be characterized adequately. Most populations had high female indices on Pickett with the exception of the race 1 populations and two of the race 5 populations (Table 2). Peking was moderately resistant or susceptible to the race 2, 4, and 9 populations evaluated. All populations except two race 9 populations had high female indices on PI88788, whereas PI90763 showed a high level of resistance to all populations not classified as race 4. Female indices on Northrup King S61-89 and Hartz 5164 were similar, although S61-89 tended to have lower female indices than Hartz 5164. The cultivar TN5-92 was distinctly different from Pickett, Northrup King S61-89, and Hartz 5164 in that it was highly to moderately resistant to

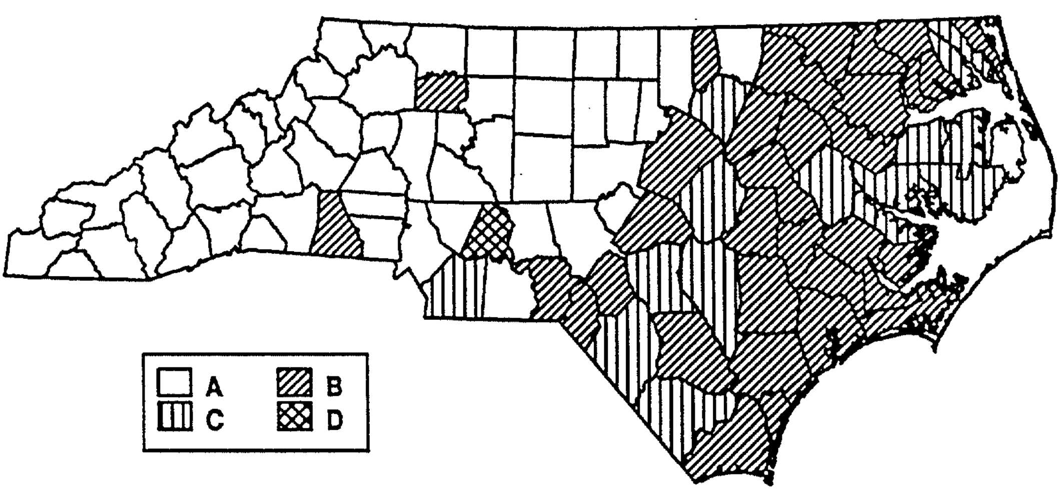

FIG. 1. County map of North Carolina. Counties not known to be infested with *H. glycines* (A,D), known infestations of *H. glycines* (B,C), and counties included in the current survey (C,D).

Table 2. Sample number, host race, mean number of *H. glycines* eggs per 500 cm^3 soil from original sample, and female indices based on susceptible cultivar 'Essex' on selected differentials used to characterize 39 of 49 field populations collected from 1994–1997.

				Female Index[a]								
Sample	Host race[b]	Eggs per 500 cm^3	Essex[c]	Pickett	Peking	PI88788	PI90763	Hartz 5164	Northrup King S61-89	TN5-92	Hartwig	C.V.[d]
1	—	200	1.3	150.0	25.0	0.0	0.0	0.0	0.0	25.0	0.0	—
28	—	4,800	12.7	231.6	31.6	105.3	15.8	236.8	168.4	36.9	0.0	—
30	—	5,600	5.3	200.0	112.5	237.5	0.0	575.0	231.2	275.0	0.0	—
48	—	1,100	10.0	126.7	53.3	46.7	13.3	80.0	26.7	53.3	6.7	—
55	—	100	6.0	61.1	27.8	50.0	5.6	133.3	166.7	55.6	0.0	—
57	—	2,150	20.0	198.3	156.7	68.4	3.0	358.3	138.3	65.0	21.7	—
59	—	1,050	19.3	39.7	39.7	41.4	5.2	32.8	108.6	10.3	0.0	—
62	—	1,800	8.0	433.3	95.8	112.5	425.0	95.8	137.5	54.2	0.0	—
64	—	1,400	20.0	66.7	15.0	38.4	6.7	65.0	41.7	10.0	0.0	—
72	—	12,100	3.5	66.7	0.0	123.8	28.6	76.2	114.3	19.0	0.0	—
49	1	8,400	326.7	2.9	0.0	20.8	0.0	41.2	11.2	0.0	0.0	122.2
58	1	300	32.7	1.0	2.0	57.1	0.0	17.3	50.0	19.4	0.0	80.9
2	2	7,500	397.3	65.6	19.6	45.5	1.9	53.2	89.6	18.3	0.0	95.8
3	2	15,500	154.7	18.5	44.0	24.6	4.7	28.0	45.7	25.0	0.0	90.8
5	2	18,500	165.3	92.3	33.5	39.1	0.2	139.5	122.6	28.2	0.0	132.1
9	2	7,600	72.7	77.1	21.1	61.5	5.5	86.7	55.0	47.7	4.6	77.4
10	2	8,100	104.0	75.0	29.5	22.4	3.2	128.8	116.7	46.1	0.6	84.5
12	2	28,300	98.0	63.3	36.0	15.0	0.0	31.3	17.0	7.5	0.0	63.3
13	2	300	45.3	60.3	20.6	72.1	0.0	222.1	133.8	4.4	1.5	85.2
14	2	900	56.0	169.1	52.4	95.2	3.6	182.1	126.2	41.7	0.0	34.3
15	2	2,500	152.7	79.5	24.9	34.9	1.8	95.2	47.6	53.7	0.0	95.1
16	2	6,600	81.3	165.6	40.2	27.0	3.3	127.9	66.4	47.5	0.0	127.2
17	2	100	110.7	112.1	35.5	21.7	3.6	74.7	84.9	43.4	0.0	61.3
19	2	36,300	122.0	78.1	33.9	36.1	1.6	160.1	49.2	7.6	0.5	86.2
25	2	400	192.0	13.2	12.9	28.1	2.4	63.9	54.9	15.2	0.0	70.7
29	2	7,500	56.7	61.2	44.7	14.1	0.0	102.3	43.5	35.3	0.0	94.0
31	2	6,200	51.3	376.6	11.7	14.3	0.0	102.6	92.2	46.7	1.3	139.5
32	2	4,000	63.3	214.7	10.5	27.4	0.0	143.2	7.4	14.3	1.6	162.9
35	2	4,300	78.0	91.5	10.3	29.9	4.3	147.0	122.2	56.4	3.4	159.3
50	2	12,200	111.3	101.8	22.2	29.9	1.8	33.5	83.2	18.0	0.0	80.9
51	2	8,600	206.0	148.9	55.7	60.2	2.6	84.1	108.4	45.6	0.0	54.9
54	2	300	49.7	30.9	14.1	32.9	8.0	37.6	40.9	47.6	0.0	45.3
60	2	400	47.7	88.1	75.5	37.1	4.9	58.0	17.5	68.5	0.7	79.6
66	2	10,200	33.3	85.0	29.0	29.0	0.0	57.0	88.0	16.0	0.0	91.9
6	4	36,500	231.3	104.3	63.7	34.0	14.7	95.4	63.1	22.5	0.0	64.0
7	4	5,300	48.0	106.9	51.4	48.6	15.3	70.8	98.6	30.6	0.7	50.7
11	4	19,900	46.0	98.6	76.8	97.1	15.9	139.1	89.9	26.1	0.0	106.8
23	4	300	62.0	114.0	36.6	75.3	12.9	64.5	61.3	23.7	1.1	92.1
26	4	3,700	53.3	148.8	61.2	65.0	12.5	130.0	75.0	42.5	1.2	35.9
33	4	4,700	94.0	136.2	22.7	21.3	28.4	130.5	81.6	12.8	0.0	95.6
26	4	2,000	99.3	49.7	16.1	20.8	20.1	33.6	10.1	2.5	1.3	120.5
61	4	350	39.7	89.1	71.4	69.8	36.1	36.1	68.9	105.0	0.0	58.0
77	4	10,800	36.3	117.4	64.2	67.0	61.5	50.5	59.6	37.6	2.7	78.9
4	5	900	42.7	68.8	4.7	17.2	0.0	48.4	42.2	20.3	1.6	176.7
8	5	7,000	84.0	18.3	2.4	84.9	3.2	172.2	135.7	1.6	0.0	97.0
52	5	19,100	167.3	34.3	4.0	42.2	3.2	153.0	51.8	3.6	0.0	169.4
67	5	4,000	68.3	67.8	8.3	44.9	1.0	89.3	56.6	4.9	0.0	90.8
34	9	18,900	98.0	81.6	12.2	9.5	0.0	129.9	115.6	13.6	0.0	158.5
37	9	5,800	110.0	90.9	15.8	8.5	0.6	57.6	37.6	6.7	0.0	101.4
Mean				128.5	39.4	53.9	8.0	132.1	85.4	40.1	1.0	
LSD ($P < 0.05$)				274.6	20.5	100.3	20.5	241.7	134.6	135.9	6.1	

[a] Female index equals 100 times the number of females on soybean differential divided by the number of femles on Essex and is mean of 3 replications.

[b] Race designation according to Riggs and Schmitt, 1988.

[c] Values for Essex are actual number of cysts on Essex.

[d] C.V. is coefficient of variation.

all race 1, 5, and 9 populations. Furthermore, races 2 and 4 had consistently lower female indices on TN5-92 than on the other cultivars tested. Hartwig was highly resistant to all populations characterized to race, with female indices ranging from 0 to 4. Insufficient numbers of *H. glycines* were extracted from six of the samples to permit accurate bioassays. An additional 10 samples produced fewer than 30 cysts on Essex soybean, and this was judged insufficient to characterize the host race, although the data are presented (Table 2).

All soybean fields sampled contained *Helicotylenchus* spp. (Table 3). *Tylenchorhychus* spp., detected in 77% of fields, were also very common. Species of *Pratylenchus* were common, occurring in 72% of the sites sampled, and included *P. brachyurus, P. penetrans, P. scribneri,* and *P. zeae.* Only one field, in Washington County, was infested with *P. penetrans,* whereas *P. brachyurus* and *P. zeae* were more common, often occurring in the same field. Numbers of *Meloidogyne* spp. ranged up to 1,000/500 cm^3 soil in 20% of the fields sampled. Only *M. incognita* was positively identified from samples. Less commonly detected nematodes included *Paratrichodorus minor, Xiphinema* spp. (*X. americanum* primarily), *Mesocriconema* spp. (*M. ornatum* most frequently). *Hoplolaimus galeatus* and *H. columbus* were detected, but the latter occurred at only one site and in low numbers.

TABLE 3. Incidence of plant-parasitic nematodes from 77 soybean fields in North Carolina, from samples collected in September of each year from 1994–1996.

Taxon	Frequency (percent)	Number per 500 cm^3 soil: Mean	Number per 500 cm^3 soil: Maximum
Helicotylenchus spp.	100	245	13,284
Heterodera glycines			
Cysts	71	11	502
Eggs	68	894	36,500
Juveniles	60	29	1,240
Haplolaimus galeatus	19	13	485
H. columbus	1	1	80
Meloidogyne spp.	26	16	1,000
Mesocriconema spp.	22	15	580
Paratrichodorus spp.	56	4	220
Pratylenchus spp.	94	27	1,355
Tylenchorhynchus spp.	81	22	825
Xiphinema spp.	21	6	70

Data are means of two samples per field.

Discussion

The frequency of occurrence of soybean cyst nematode races found in this survey cannot be compared meaningfully with random surveys previously conducted in North Carolina (Schmitt and Barker, 1987). A justifiable bias was introduced into the current survey to optimize sampling time and because the primary objective was determining the race status of North Carolina *H. glycines* populations.

In contrast to our findings, races 9 and 14 were the most frequently detected in South Carolina (Lewis et al., 1993). These data also differ greatly from a previous survey in which races 1, 2, 3, 4, and 5 comprised 77% of populations evaluated in North Carolina in 1985–1986 (Schmitt and Barker, 1987). Race 2 of *H. glycines,* which was the most prevalent in the earlier study and the current work, increased from 21% to 45% of the soybean cyst nematode populations, whereas race 4 increased from 7% to 17% over a span of approximately 10 years. The differences between North Carolina and South Carolina *H. glycines* populations can probably be attributed to three factors: (i) more intensive soybean culture in North Carolina, (ii) genetic differences in populations as a result of limited diversity in new infestations, (iii) different cultivar usage between the two states.

No populations that could be categorized as race 3 or 14 of *H. glycines* were detected in the current study. Similarly, race 3 was detected in only one field in Tennessee, which borders both North Carolina and Missouri (Young, 1990). In contrast, race 3 is the most common race of this nematode found in Missouri, Ohio, and Illinois (Niblack et al., 1993; Sikora and Noel, 1991; Willson et al., 1996). Still, 16 *H. glycines* populations found in the current survey were not classified as to race. Some of these populations could be races 1, 3, or 14. Five of these populations came from Franklin County in the

Piedmont from fields in a tobacco-soybean-maize rotation that incorporated soybean cyst nematode-resistant cultivars. Many of the low-population densities of soybean cyst nematode in the samples were probably the result of the use of resistant cultivars. However, the majority of the remaining 11 populations not categorized because of low reproduction on Essex had adequate reproduction on Pickett, suggesting they probably were not races 1 or 3.

The high level of reproduction of *H. glycines* on Northrup King S61-89 and Hartz 5164 indicates that the usefulness of this type of resistance in North Carolina at this time is limited. These cultivars, though varying from moderately resistant to susceptible to races 2 and 4, generally yield more in field trials than susceptible cultivars or race 1 and race 3-resistant cultivars (S. R. Koenning, unpublished). An important facet of a rotation program incorporating resistant and susceptible cultivars is the ability of the resistant cultivar to limit reproduction of soybean cyst nematode such that a subsequent susceptible cultivar may be planted. Cultivars moderately resistant to a given population may fail to limit *H. glycines* population levels to non-damaging levels in North Carolina, even though yield is not seriously affected in the current year. Thus, a management strategy of rotating resistant and susceptible cultivars currently has limited value in North Carolina. Certainly, *H. glycines* race 1- and race 3-resistant cultivars should generally be considered as susceptible to *H. glycines* in North Carolina. Cultivars with resistance similar to TN5-92 or Cordell would be useful in managing some race 2, 4, or 5 populations, but these cultivars have not become popular in the state. Cultivars with the broad resistance from PI437654 are needed to manage the majority of soybean cyst nematode populations in North Carolina. Young (1990) also found a large proportion of *H. glycines* populations in Tennessee that could be classified as races 2, 4, or 5. In general, data from studies through the United States indicate that populations of *H. glycines* continue to shift in response to selection pressure as a result of the deployment of resistant cultivars. The high percentage of races 2, 4, 5, 9, and 14 in the southeastern United States compared to the prevalence of race 3 in northern states is probably a result of the long history of using resistant soybean cultivars in the southeastern United States. Populations of *H. glycines* from the southeastern United States thus have a higher frequency of alleles for parasitism on resistant soybean than those in the northern states.

Considerable variation occurred between *H. glycines* populations classified as races 2 or 4 with regard to parasitism on PI88788, TN5-92, Hartz 5164, and Northrup King S61-89. These results verify the conclusions of Riggs et al. (1995) that a new scheme for classification of *H. glycines* host races is needed. The cultivar TN5-92, with a background similar to Cordell, appears to be distinctly different from other cultivars.

Only 33% of North Carolina (Schmitt and Barker, 1987) and 14% and 17% of South Carolina fields in earlier surveys (1985–1989) were infested with *H. glycines* (Lewis et al., 1993). In the current survey, 71% of the fields sampled had detectable levels of *H. glycines.* If only fields in counties in the Coastal Plain and Tidewater regions of North Carolina are considered, then 80% of the fields in these regions are infested. This conclusion may be an overestimation of the problem since fields were not selected at random for this survey. Bias also may have been introduced by the fact that counties with a large soybean hectarage were chosen, since the primary objective of this study was to assess the race status of *H. glycines* populations. Furthermore, Extension agents were asked to locate fields for which information on past production practices could be obtained, which may have been another source of bias.

The two counties in which this nematode was not detected, Union and Stanly counties, are both in the Piedmont, which is characterized by rolling hills and fine-textured soils. The majority of the fields in these two counties have been in no-till for 5 or more years in a 1-year corn-soybean rotation. Although population densities of *H. glycines* tend to be suppressed in no-till (Koenning et al., 1995), we suspect that other factors have limited the spread of this nematode in the Piedmont. Farm equipment such as planters, tractors, and cultivators used on fine-textured soils and rolling hills in the Piedmont are not adapted to sandy coastal plain soils. Thus, the likelihood of movement of soybean cyst nematode on machinery from the coastal plain to the Piedmont is small. Secondly, distances between fields tend to be greater in the Piedmont than on the coastal plain, thus limiting spread within the Piedmont. Previously, soybean cyst nematode had been detected in Union County but not in Stanly County (D. P. Schmitt, personal communication).

The relative frequency of occurrence of the various taxa of plant-parasitic nematodes on soybean generally concurs with published reports from North Carolina and South Carolina (Lewis et al., 1993; Schmitt and Barker, 1987). The incidence of *Meloidogyne* spp. was much lower than is usually encountered in other crops in North Carolina, especially vegetables, cotton, and tobacco (K. R. Barker and J. L. Imbriani, personal communication). High population densities of *H. glycines* may competitively exclude *Meloidogyne* spp. in soybean fields. Also, most currently grown soybean cultivars are either resistant or poor hosts for *M. incognita*. *Hoplolaimus columbus*, detected in only one field, and the reniform nematode, *Rotylenchulus reniformis*, not detected in this survey, are believed to occur in only a few North Carolina counties at this time.

In summary, a large percentage of the *H. glycines* populations detected in this survey cannot be managed adequately by currently available host resistance, with the exception of two cultivars, Hartwig and TN5-92. More effort is needed to incorporate these types of resistance in high-yielding soybean.

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