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Tolerance to *Hoplolaimus columbus* in Glyphosate-Resistant, Transgenic Soybean Cultivars[1]

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Abstract: Transgenic soybean cultivars, resistant to glyphosate herbicide in maturity groups V and VI, were evaluated for tolerance to the Columbia lance nematode, *Hoplolaimus columbus,* in field experiments conducted in 1998 and 1999. Treatment with 42 liter/ha of 1,3-dichloropropene was effective in suppressing *H. columbus* population densities in a split-plot design. Fumigation increased soybean yield, but a significant cultivar × fumigation interaction indicated variation in cultivar response to *H. columbus.* A tolerance index (yield of nontreated ÷ yield of treated × 100) was used to compare cultivar differences. Two cultivars in maturity group VI and one cultivar in maturity group V had a tolerance index greater than 90, indicating a high level of tolerance.

Key words: Columbia lance nematode, crop loss, fumigant nematicide, *Glycine max,* glyphosate, herbicide-resistant crops, *Hoplolaimus columbus,* host-plant tolerance, nematode, soybean.

The Columbia lance nematode, *Hoplolaimus columbus* Sher, is limited in known distribution to Georgia, North Carolina, South Carolina, and Alabama in the United States (Koenning et al., 1999). In areas where this nematode occurs, it can be a devastating pathogen of cotton, *Gossypium hirsutum* L., corn, *Zea mays* L., and soybean, *Glycine max* L., especially in sandy soils (Kinloch, 1998; Koenning et al., 1990; Lewis and Smith, 1976; Noe, 1993; Nyczepir and Lewis, 1979; Perez et al., 1996; Schmitt and Bailey, 1990). Tactics for management of this plant-parasite are limited. Rotation is often not an option in fields infested with *H. columbus* due to its wide host range (Fassuliotus, 1974). Peanut (*Arachis hypogaea* L.) and tobacco (*Nicotiana tabacum* L.) can be used in rotation with host crops, but the hectarage of and demand for these two crops are limited. Winter wheat or rye cover crops had no impact on population densities of *H. columbus* (Davis et al., 2000). Fumigant and nonfumigant nematicides are effective in preventing soybean and cotton yield suppression by this nematode (Mueller and Sanders, 1987; Mueller and Sullivan, 1988; Noe, 1990; Perez et al., 1996; Schmitt and Imbriani, 1987). The use of nematicides on soybean, however, is limited because of the low value of soybean per hectare. Subsoiling in soils with a hardpan has increased soybean yield when *H. columbus* is present, but many farmers have adopted reduced tillage practices (Hussey, 1977; Schmitt and Bailey, 1990). Late planting of soybean proved to be an effective means of alleviating soybean yield suppression by the nematode pathogens *Pratylenchus brachyurus* and *Heterodera glycines* (Koenning et al. 1985; Koenning et al., 1993), but not for *H. columbus* (Perez et al., 1996).

Soybean cultivars vary in their response to parasitism by nematodes. Resistant cultivars are generally defined as limiting the reproduction of a particular nematode species or race, whereas susceptible cultivars support large numbers of the nematode parasite in question. Little variation between soybean and(or) cotton cultivars to support reproduction of *H. columbus* has been noted, which may indicate that there is no specific host-plant resistance to this nematode (Bowman and Schmitt, 1994; Mueller et al., 1988). Soybean cultivars differ in their sensitivity to *H. Columbus,* as measured by growth and yield in the presence of the pathogen, and this variation between cultivars has been referred to as tolerance (Nyzepir and Lewis, 1979; Mueller et al., 1988; Mueller and Sanders, 1987; Schmitt and Imbriani, 1987). Cultivars that support nematode reproduction, but are little affected in productivity, are referred to as being tolerant (Barker, 1993; Cook and Evans, 1987). Soybean tolerance to a number of nematodes, including *H. glycines* and *H. columbus,* has been documented (Boerma et al., 1986; Mueller and Sullivan, 1988; and Schmitt and Imbriani, 1987). Information on cultivar resistance to soybean cyst and root-knot nematodes may be obtained through greenhouse experiments, but tolerance must generally be evaluated through field experimentation (Bowman and Schmitt, 1994; Mueller et al., 1988).

The widespread use of herbicide-resistant soybean cultivars has resulted in many soybean cultivars being relatively obsolete. Currently, approximately 80% of the soybean hectarage in North Carolina is planted to transgenic cultivars (Roundup Ready, Monsanto Corp. St. Louis, MO) resistant to the herbicide glyphosate (Dunphy, pers. comm.). Data on field tolerance and(or) resistance of transgenic cultivars to pests and pathogens is generally lacking. Field research conducted in 1998 and 1999 evaluated the tolerance of glyphosate-resistant soybean cultivars to the Columbia lance nematode. The objectives of this research were to evaluate the impact of this nematode on seed yield of selected maturity group V and VI transgenic soybean cultivars, and the reproductive capacity of *H. columbus* on these cultivars.

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Materials and Methods

Two Þeld experiments were conducted, one in 1998 and a second in 1999. Both experiments were located in Scotland County, North Carolina, in Þelds naturally infested with *H. columbus* that had previously been planted with cotton. The soil types were a Norfolk loamy sand (84% sand, 14% silt, 2% clay; <1% organic matter) in 1998, and a Wagram loamy sand (84% sand, 11% silt, 5% clay, <1% organic matter) in 1999. Mean pre-fumigant population densities of *H. columbus* in the Þeld sites were 425 ± 131, 395 ± 60 per 500 cm^3 soil in 1998 and 1999, respectively. The experimental design was a split-plot with six replicates. Sub-plots were treated with 42 liter/ha of 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN) vs. nontreated. Whole plots were transgenic glyphosate-resistant cultivars AG 6101 (Monsanto, St. Louis, MO), DP 6880RR (Delta and Pine Land, Scott, MS), and S 60E44RR (Syngenta Seeds, Inc., Minneapolis, MN) in maturity group VI and AG 5602, AG 5801, DP 5644RR, DP 5806RR, DP 5960RR, and S57-A4 in maturity group V.

Selected plots were fumigated in April and all plots planted in mid-May. Plots were four rows 7.62 m long with 1.01-m row spacing and 3.0-m alleys. Soybean seed yield was determined using a research combine in mid-November. Samples for nematode assays for each plot were collected prior to fumigation and at soybean harvest. Each soil sample consisted of 8 to 10 cores (2.5-cm-diam.) taken to a depth of 15 cm and composited. A 500-cm^3 sub-sample was processed by elutriation and centrifugation to extract adult and juvenile nematodes from soil. Roots were collected from a sieve on the elutriator and placed in a mist extractor for 5 days to collect vermiform stages (Barker et al., 1986).

Data analysis consisted of analysis of variance for a split-plot design using PC/SAS software (SAS Institute, Cary, NC). Locations for the Þeld tests were considered to be random effects so the cultivar × location was used to test differences for cultivar, fumigation, and cultivar × fumigation effects, respectively, for combined analysis over years. Because location had no signiÞcant effect on yield or nematode numbers, pooled data from the two locations are presented (Table 1). Tolerance indices (TI) were calculated, where TI = yield of nontreated Ö yield of treated × 100. The least signiÞcant difference test (LSD) was used to separate cultivar means if main effects from the analysis of variance indicated signiÞcance at $P < 0.10$. Orthogonal contrasts were used to compare maturity group V vs. maturity group VI cultivars.

Results and Discussion

Fumigation with 1,3-dichloropropene was effective in suppressing the population density of *H. columbus* ($P = 0.05$) at soybean harvest (Table 1). Because of its relatively low rate of reproduction on soybean compared to other plant-parasitic nematodes such as root-knot and cyst nematodes, *H. columbus* cannot compensate for the initial kill by a fumigant. Cultivars did not differ in their ability to support reproduction of *H. columbus*. This conclusion agrees with previous research, indicating that little variation in soybean cultivars to support *H. columbus* reproduction exists (Mueller et al., 1987; Nyczepir and Lewis, 1979; Schmitt and Imbriani, 1987). The later-maturing cultivars had somewhat higher population densities of *H. columbus* than did maturity group V cultivars, although this difference was not statistically signiÞcant. Later-maturing cultivars may sup-

Table 1. Inßuence of fumigation with 42 liter/ha of 1,3-dichloropropene on soybean yield (kg/ha), tolerance index (TI), and harvest population densities of *Hoplolaimus columbus*/500 cm^3 soil on nine glyphosate-resistant, transgenic soybean cultivars tested in 1998 and 1999 in Scotland County, North Carolina.

Cultivar	Maturity group	Yield + Fumigant	Yield • Fumigant	TI[a]	No. lance • Fumigant	No. lance + Fumigant
AG 6101	VI	2,353	2,279	97.1	359	118
DP 6880RR	VI	2,597	2,455	96.7	405	163
S 60E44RR	VI	2,315	1,614	82.4	522	183
Mean Group VI[b]		2,422	2,116	92.1	428	155
AG 5602	V	2,260	1,570	75.9	343	132
AG 5801	V	2,429	1,708	68.2	430	88
DP 5644RR	V	2,168	2,068	96.3	448	127
DP 5806RR	V	2,536	2,063	81.6	361	169
DP 5960RR	V	2,462	1,975	81.4	348	121
S57-A4	V	2,350	1,627	68.6	418	78
Mean Group V		2,367	1,835	78.6	324	139
Mean of all cultivars[c]		2,385a	1,929b	83.1	359a	144b
LSD ($P = 0.10$) for cultivars		281	281	22.6	NS	NS

Data for each cultivar are means of 12 observations over 2 years at two sites.

[a] Tolerance indices (TI) were calculated where TI = yield of nontreated Öyield of treated × 100.

[b] Means for maturity groups do not differ according to orthogonal contrasts ($P = 0.01$).

[c] Means in row followed by the same letter do not differ according to the main effects analysis of variance of fumigant vs. no fumigant ($P = 0.01$); the cultivar × fumigation interaction was signiÞcant for soybean yield ($P = 0.0345$).

port additional nematode reproduction due to the longer growing season, as may occur with *H. glycines* (Koenning et al., 1993).

Fumigation increased the yield of soybean compared to nonfumigated plots (Table 1). The Þrst-order cultivar × fumigation interaction was signiÞcant ($P = 0.035$), indicating that the seed yield of cultivars differed in response to infection by *H. columbus.* Other Þrst- and second-order interactions were not signiÞcant. Maturity group VI cultivars tended to yield slightly more than the maturity group V cultivars. An adequate comparison of maturity groups with respect to yield, however, is not possible due to the limited number of group VI cultivars tested.

Tolerance indices for the cultivars ranged from 68 to 97, demonstrating that considerable variation in tolerance to *H. columbus* exists in herbicide-resistant cultivars. This range of tolerance is similar to that reported previously, but no cultivar studied in the current research was as intolerant as DP 105 (TI = 12.0) (Schmitt and Imbriani, 1987). Three cultivars, AG 6101, DP 6880RR, and DP 5644RR, had tolerance indices greater than 90, which would be a convenient reference for cultivar selection. The yield of DP 5644RR, however, was among the lowest of all cultivars tested in fumigated plots. The yield potential of cultivars should be considered in decisions on cultivar selection. Still, assuming a price of US$184.00/metric ton of soybean and the cost of a minimal rate of 1,3-dichloropropene at US$48.00/ha (14.0 liters/ha @ US$3.42/liter), the gross revenue/ha for cultivars with a TI greater than 90 not treated with a nematicide was US$379.00ÐUS$451.00 compared to US$361.00ÐUS$418.00 for cultivars with a TI less than 90 after subtracting the cost of 1,3-dichloropropene. The greater percentage of tolerant cultivars in Maturity group VI compared to maturity group V may be due to the low number of cultivars tested or more selection by plant breeders for tolerance in later-maturing cultivars.

The use of cultivars tolerant to *H. columbus* is a viable option for growers when this nematode is present at damaging levels. Tolerance to nematodes, however, is density dependent (Boerma et al. 1986; Koenning et al., 1992). Additional tactics may be required to manage *H. columbus* if population densities greatly exceed the damage threshold. Research comparing cultivar response to *H. columbus* at varying population densities is needed, but difficult to achieve in practice. Evaluation of tolerance at different population levels requires extensive land and material for testing (Koenning et al., 1992). Comparison of fumigated vs. nonfumigated plots appears to be the most cost-effective means for evaluating tolerance (Bowman and Schmitt, 1994; Reese et al., 1988). Expanded screening of soybean, cotton, and corn germplasm may identify resistance or greater levels of tolerance to this nematode. The impact of cropping systems, cover crops, and potential changes in cultural practices on *H. Columbus* also require investigation.

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