# RESISTANCE IN MAIZE TO *PARATRICHODORUS MINOR*

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## ABSTRACT

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The stubby-root nematode *Paratrichodorus minor* is widespread in the southeastern United States. Maize is both a good host for reproduction and sensitive to feeding damage from this nematode. The primary objective of this study was to evaluate commercial maize hybrids and public inbred lines for resistance to *P. minor*. A secondary objective was to determine whether there was a correlation between nematode reproduction and the concentration of benzoxazinones in maize roots. Thirty three commercial maize hybrids and 22 public inbred lines were evaluated for resistance to *P. minor* in the greenhouse. Pioneer hybrid 3223 served as a susceptible control. Pots containing sterilized loamy sand and one maize plant were inoculated with 500 mixed vermiform stages. Nematodes were extracted from the soil approximately 50 days after inoculation. Four hybrids (Croplan 822RR2/Bt, Southern States 842RR, Garst 8200YGI, and Pioneer 31G98) were moderately resistant to *P. minor* in three trials. Nematode reproduction on these four hybrids averaged less than 50% the reproduction on Pioneer 3223. None of the public inbred lines tested showed any resistance to *P. minor* relative to Pioneer 3223. The benzoxazinones present in the maize roots were the MBOA aglycone, and glucosides of $DIM_2BOA$ and DIMBOA. Concentrations of total benzoxazinones differed among maize hybrids; however, there was no relationship between concentrations and reproduction of *P. minor*. To better predict resistance to *P. minor* in maize hybrids, additional research is needed to identify inbred lines with resistance. Discovery of chemical or genetic markers for resistance would also be useful for screening inbred lines and hybrids.

*Key words:* Benzoxazinones, DIMBOA, hydroxamic acids, maize, *Paratrichodorus minor*, resistance, stubby-root nematode, *Zea mays*.

## RESUMEN

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*Paratrichodorus minor* se encuentra ampliamente distribuido en el sureste de los Estados Unidos. El maíz es un buen hospedante y sensible al daño ocasionado por este nematodo. El objetivo prinicipal de este estudio fue evaluar la resistencia a *P. minor* en híbridos comerciales de maíz y líneas endógamas de dominio público. Un objetivo secundario fue determinar si existe correlación entre la reproducción del nematodo y la concentración de benzoxazinonas en las raíces del maíz. Se evaluó la resistencia a *P. minor* en 33 híbridos comerciales de maíz y 22 líneas endógamas de dominio público en invernadero. Se utilizó el híbrido 3223 de Pioneer como control susceptible. Se inocularon macetas con arena limosa esterilizada y una planta de maíz con 500 formas vermiformes mixtas del nematodo. Se extrajeron los nematodos presentes en el suelo aproximadamente 50 días después de la inoculación. Cuatro híbridos (Croplan 822RR2/Bt, Southern States 842RR, Garst 8200YGI y Pioneer 31G98) fueron moderadamente resistentes a *P. minor* en tres ensayos. La reproducción del nematodo en estos cuatro híbridos fue, en promedio, menor del 50% de la reproducción observada en Pioneer 3223. Ninguna de las líneas endógamas evaluadas mostró resistencia a *P. minor* en comparación con Pioneer 3223. Las benzoxazinonas presentes en las raíces del maíz fueron aglicona MBOA, y glucósi-

dos de $DIM_2BOA$ y DIMBOA. Aunque se encontraron diferencias en las concentraciones totales de benzoxazinonas de los híbridos de maíz; no se encontró correlación entre estas concentraciones y la reproducción de *P. minor*. Para poder predecir la resistencia a *P. minor* en híbridos de maíz, se requiere más investigación para identificar líneas endógamas con resistencia. También sería útil el descubrimiento de marcadores genéticos o químicos para indicar resistencia en líneas e híbridos.

*Palabras claves:* Benzoxazinonas, DIMBOA, ácidos hidroxámicos, maíz, *Paratrichodorus minor*, resistencia, *Zea mays*.

## INTRODUCTION

The stubby-root nematode *Paratrichodorus minor* is widespread in the southeastern United States (Perry and Rhoades, 1982). In Georgia, *Paratrichodorus* spp. were found in 31% of 102 corn fields sampled (Davis and Timper, 2000). Although other crops are hosts, maize (*Zea mays*) is the primary field crop damaged by this nematode (Koenning *et al.*, 1999; Perry and Rhoades, 1982; Rohde and Jenkins, 1957; Schilt and Cohn, 1975). Maize is both a good host for *P. minor* reproduction and sensitive to feeding damage from the nematode (Christie and Perry, 1951). The nematode aggregates and feeds in the zone of root elongation and the apical meristem causing root growth to slow and eventually stop (Schilt and Cohn, 1975). Seedlings are especially vulnerable to damage from *P. minor* because they have small, developing root systems. If nematode populations are suppressed during the early stages of maize growth, then the plant can establish a good root system and becomes more tolerant of the nematode.

*Paratrichodorus minor* has the capacity for extremely rapid population increase. Schilt and Cohn (1975) reported over 200-fold increase of the nematode after 30 days on eggplant. Timper and Hanna (2005) reported similar increases after 60 days on maize, pearl millet (*Pennisetum glaucum*), and cotton (*Gossypium hirsutum*). Maize hybrids that support less reproduction of *P. minor* (i.e., resistant hosts) may experience less damage than hybrids that support more reproduction (i.e., susceptible hosts). The prospect of identifying resistance to *P. minor* in maize is bolstered by the recent discoveries of host-plant resistance to this nematode in other members of Poaceae. In tall fescue (*Festuca arundinacea*), the presence of the endophyte *Neotyphodium coenophialum* had no effect on population densities of *P. minor*; however, nematode densities in soil planted to 'Jesup' were <5% of the densities in soil planted to 'Georgia 5' (Timper and Bouton, 2004). In both field and greenhouse experiments, some pearl millet hybrids (including 'TifGrain 102') were more resistant to *P. minor* than HGM-100 (Timper and Hanna, 2005; Timper *et al.*, 2002).

The primary objective of this study was to evaluate commercial maize hybrids and public inbred lines for resistance to *P. minor*. We determined resistance based on the ability of the plant to suppress nematode reproduction relative to a susceptible standard (Pioneer 3223). A secondary objective was to determine whether there was a correlation between nematode reproduction and the concentration of benzoxazinones in maize roots. Benzoxazinones are produced by a number of plants in the Poaceae and have toxicity against a broad spectrum of organisms (Friebe, 2001). These compounds are found in both foliage and root tissue, and their concentrations can vary among plant genotypes. The predominant benzoxazinone in maize, DIMBOA [2,4-hydroxy-7-methoxy-(2H)-1,4-

benzoxazin-3-(4H)-one], is involved in resistance to several insect species, including root-feeding insects (Friebe, 2001). For example, survival and growth parameters of the western corn rootworm were negatively correlated with concentrations of DIMBOA in maize roots (Xie *et al.*, 1992). Zasada *et al.* (2005) recently showed that DIMBOA and DIBOA were toxic to some plant-parasitic nematodes *in vitro*. However, it is unknown whether DIMBOA or other benzoxazinones are involved in host-plant resistance to nematodes.

## MATERIALS AND METHODS

### *Nematode Reproduction on Hybrids and Inbreds*

The commercial maize hybrids evaluated in the study are listed in Table 1. The hybrids represent a diverse selection of maize adapted to the southeastern United States. Initially, the hybrids were tested in groups of 11 entries in three separate experiments (Experiments 1, 2, and 3). Following these initial experiments, ten hybrids were selected based on high and low nematode reproduction and re-evaluated in a fourth experiment. Experiment 4 was performed twice. In each experiment, Pioneer 3223 served as a susceptible control. This hybrid was previously shown to be a good host for *P. minor* (Timper and Hanna, 2005). Two seeds of each hybrid were planted in 15-cm-diam plastic pots containing 2,700 $cm^3$ of loamy sand (82% sand, 9% silt, 7% clay, 1% organic matter; pH 5.3) that had been steam heated at 100°C for 6 h to kill potential plant pathogens. After germination, plants were thinned to one per pot. There were five replicate pots for each entry and these were completely randomized on a single greenhouse bench. The *P. minor* used in the experiments was isolated from tall fescue in Clarke Co., GA, and cultured on tall fescue and St. Augustine grass (*Stenotaphrum secundatum*). Inoculum of *P. minor* was extracted from soil using centrifugal flotation (Jenkins, 1964). The maize plants were inoculated with 500 mixed vermiform stages by pipetting the nematodes in an aqueous solution onto the soil surface 11 to 20 days after planting. A drip system was used to irrigate the plants twice a day. Plants were fertilized at planting with a slow release formulation (14-14-14, N-P-K). Soil temperatures in the greenhouse ranged from 17 to 35°C. Nematodes were extracted from 250 $cm^3$ of soil from each pot 49 to 54 days after inoculation using centrifugal flotation.

The public inbred lines (Table 2) were initially tested in groups of 11 entries in two separate experiments (Experiment 5 and 6). Following the initial experiments, 10 inbred lines were selected for re-evaluation (Experiment 7). To determine whether hybrids were more resistant to *P. minor* than the inbred lines (heterosis), an additional experiment was conducted (Experiment 8) in which eight inbreds and six hybrid combinations of the inbred lines were evaluated for nematode reproduction. Pioneer 3223 served as the susceptible control in all experiments; in Experiment 8, Va35 served as an additional susceptible control. Experiments 7 and 8 were performed twice. The methods for the inbred experiments were the same as the hybrid experiments except that plants were inoculated with nematodes 17 to 29 days after planting.

### *Concentration of Benzoxazinones in Maize Roots*

The maize hybrids evaluated in Experiment 4 and the inbred Va35 were planted one seed per pot in heat-treated soil. After 3 weeks of growth, the root systems of three plants per entry were rinsed with tap water, dried with a paper towel, weighed,

Table 1. Commercial maize hybrids used in the study and their characteristics.

| Company | Hybrid | Transgene(s) |
|---|---|---|
| Grabow Seed, Dunwoody, GA | AT 221BtRR | *Bt* Corn Borer, Roundup Ready |
| | AT 755RR | Roundup Ready |
| | AT 855RR | Roundup Ready |
| Croplan Genetics, Midland City, AL | Croplan 799Bt | *Bt* Corn Borer |
| | Croplan 822RR2/Bt | *Bt* Corn Borer, Roundup Ready |
| | Croplan 851RR2/Bt | *Bt* Corn Borer, Roundup Ready |
| Monsanto Global Seed Group, Dekalb, IL | DKC 67-60 | Roundup Ready |
| | DKC 68-70 | *Bt* Corn Borer |
| | DKC 69-70 | *Bt* Corn Borer |
| United Agri Products, Kingston, AL | Dynagro 5518 | |
| | Dynagro 58K22 | Roundup Ready |
| Garst Seed Company, Slater, IA | Garst 8200YGI | *Bt* Corn Borer |
| | Garst 8222IT | |
| | Garst 8288 | |
| Golden Acres, Chester, GA | GA 7885 | |
| | GA 7997 | |
| | GA 8112 | |
| Greenwood, Laurel Hill, FL | Greenwood 860 | |
| Pioneer Hi-bred International, Huntsville, AL | P 30F34 | *Bt* Corn Borer |
| | P 31G66 | |
| | P 31G98 | |
| | P 32D99 | |
| | P 32W86 | |
| | P 33H25 | |
| Southern States Co-op, Richmond, Va | SS 02-201 | |
| | SS 781CL | Clearfield |
| | SS 842RR | Roundup Ready |
| Syngenta Seeds, Winterville, N.C. | NK 1851W | |
| | NK N83-N5 | |
| | NK N91-R9 | |
| Terral Seed, Inc., Lake Providence, La | TV2140Xn1RR | Roundup Ready |
| | TV2160Bt | *Bt* Corn Borer |
| Royster-Clark, Inc., Norfolk, Va | V61R36 | Roundup Ready |

and cut into 1-cm pieces. A 0.3-g subsample of each root system was placed in a 2-ml microcentrifuge tube and macerated with a pestle. Distilled water (0.5 ml) was added to the tube and the sample was incubated at room temperature for 30 minutes (Davis *et al.*, 2000). After incubation, 0.5 ml of methanol was added to the tubes and agitated for 30 seconds with a vortex mixer. The samples were stored at

Table 2. Maize inbred lines used in the study, their pedigree, and developing institution.

| Inbred | Accession identifier | Pedigree and developing institution |
|---|---|---|
| B73 | PI 550473 | BSSS(S)C5—Iowa State University |
| B104 | PI 594047 | BS13(S)C5—Iowa State University |
| B110 | PI 607381 | BS13(S)C5—Iowa State University |
| B116 | PI 632746 | B97 × B99—Iowa State University |
| CML323 | Ames 27097 | Pop33 C2(STE)—CIMMYT, Mexico |
| CML326 | N/A[z] | Pop45 C6—CIMMYT, Mexico |
| CML422 | N/A | Pool17(TSR)—CIMMYT, Mexico |
| GT112 | Ames 27113 | Multiple cross including Whatley, Cuban, Garrick, Creole, and 12% other—Coastal Plain Experiment Station, Tifton, GA |
| KY21 | Ames 27130 | Boone County White—Kentucky Agricultural Experiment Station |
| Mp709 | PI 596626 | Old Raccoon (PI 540778)—USDA-ARS-Starkville, MS |
| Mp710 | PI 596627 | Old Raccoon (PI 540778)—USDA-ARS-Starkville, MS |
| Mp712 | PI 596629 | Tebeau (PI 540756)—USDA-ARS-Starkville, MS |
| Mp717 | PI 639919 | Mp420 × Tx601—USDA-ARS-Starkville, MS |
| NC258 | PI 531084 | (TZ*2/3/NC 248/NC 246)/2/C103—North Carolina State University |
| NC268 | PI 587145 | (B73 × NC250) B73—North Carolina State University |
| NC300 | Ames 27146 | Line from Pioneer X105A × a line from (Pioneer X306B × H5)—North Carolina State University |
| Tx770 | PI 633843 | (Tx601/2*Mo17)—Texas A&M University |
| Tx772 | PI 633844 | Argentine line—Texas A&M University |
| Tx807 | PI 619430 | Pob63—Texas A&M University |
| Va35 | PI 587150 | (C103 × T8)T8—Virginia Agricultural Experiment Station |

[z]Not deposited in National Seed Storage Laboratory (NSSL) germplasm collection.

0°C before quantification of benzoxazinones by High Performance Liquid Chromotography (HPLC) according to the methods of Lyons *et al.* (1988). A 50-µl aliquot of BOA (0.119 mg/50 µl MeOH) was added to each sample as internal standard and mixed. Analysis of BOA compounds was performed with a Hewlett-Packard 1090 Diode Array HPLC with UV spectral acquisition. A 20-µl aliquot of each sample was injected onto a Beckman Ultrasphere 5u C-18 4.6 × 250 mm reversed-phase column. Compounds were eluted with a linear gradient from 10% MeOH/water (solvent A) to 100% MeOH (solvent B) over 35 min (0.1% phosphoric acid was in each solvent). Flow rate was 1 ml/min and column effluent was monitored at 280 nm. HPLC-MS (mass spectrometry) analyses were performed on a Thermo-Finnigan LCQ. MS spectra were compared to those reported in the literature (Cambier *et al.*, 1999, 2000; Rice *et al.*, 2005).

*Statistical Analysis*

Number of *P. minor* per pot and fresh root weight were subjected to Analysis of Variance (ANOVA) (SAS Institute, Cary, NC). Nematode numbers were subjected

to square-root transformation prior to analysis. Fisher's LSD test was used to determine differences among entries. In experiments that were repeated in time, trial was included in the model to determine whether there was a maize entry by trial interaction. A one-way ANOVA followed by Fisher's LSD test was used to determine whether there were differences among hybrids in concentrations of benzoxazinones. Regression analysis was used to determine whether there was a relationship between reproduction of *P. minor* (mean of three trials) and concentrations of individual and total benzoxazinones.

## RESULTS

### *Nematode Reproduction on Hybrids and Inbreds*

Reproduction of *P. minor* on the susceptible control Pioneer 3223 varied among experiments. The number of nematodes per pot for Pioneer 3223 in the initial hybrid experiments was 16,200 (Pf/Pi = 32.4), 11,275 (Pf/Pi = 22.6), and 8,942 (Pf/Pi = 17.9) in Experiments 1, 2, and 3, respectively. Because the reproductive potential of *P. minor* varied among experiments, reproduction on the hybrids is presented as a percentage of Pioneer 3223. In the three initial experiments, reproduction of *P. minor* was lower ($P \leq 0.05$) on several commercial maize hybrids than on the susceptible control Pioneer 3223 (Fig. 1). No hybrid had significantly greater reproduction than the control, though several hybrids in Experiments 1 and 2 had numerically greater numbers. All hybrids in Experiment 3 had fewer *P. minor* than Pioneer 3223.

Ten commercial hybrids were re-evaluated for nematode reproduction in Experiment 4. Reproduction of *P. minor* differed between the two trials ($P < 0.0001$) and among the hybrids ($P < 0.0001$). The trends

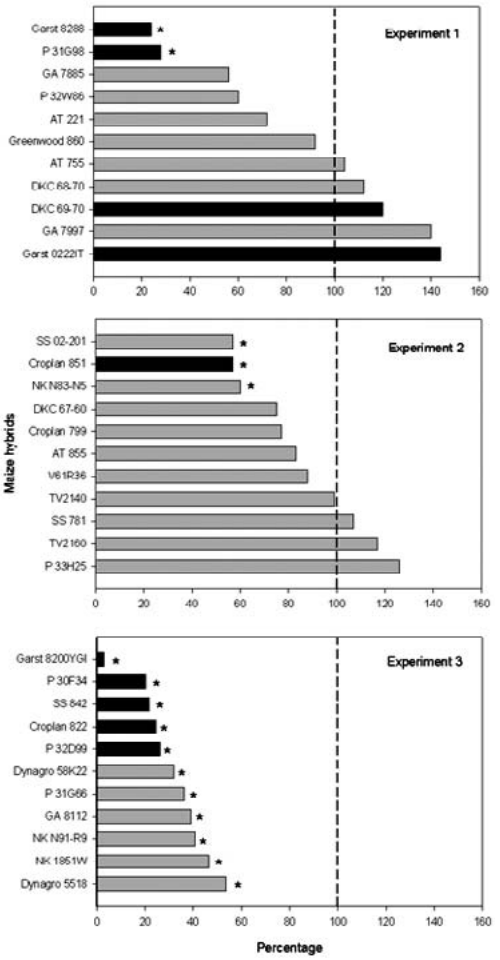


Fig. 1. Reproduction of *Paratrichodorus minor* on commercial maize hybrids as a percentage of reproduction on the susceptible control Pioneer 3223. The dashed line represents the reproduction on Pioneer 3223 which was 16,200 (nematodes per pot), 11,275, and 8,942 in Experiments 1, 2, and 3, respectively. Asterisks above bars indicate a difference ($P \leq 0.05$) from the control. The dark bars are hybrids that were re-evaluated for nematode reproduction in Experiment 4.

in reproduction among the hybrids were not consistent between the two trials ($P = 0.001$); therefore, the trials are presented separately (Table 3). Reproduction of *P. minor* was consistently lower ($P \leq 0.05$) on three hybrids (SS 842RR, Garst 8200YGI, and Pioneer 31G98) compared to Pioneer

Table 3. Reproduction of *Paratrichodorus minor* on commercial maize hybrids.

| Hybrid | Percentage of Pioneer 3223[x] | | | |
|---|---|---|---|---|
| | Exp. 1, 2 & 3 | Exp. 4, Trial 1 | Exp. 4, Trial 2 | Mean[y] |
| Garst 8222IT | 144 | 98 | 86 | 109 |
| DK69-70 | 120 | 59*[z] | 282 | 87 |
| Pioneer 32D99 | 26* | 58* | 164 | 83 |
| Croplan 851 | 58* | 92 | 68 | 72 |
| Garst 8288 | 24* | 113 | 36* | 57 |
| Pioneer 30F34 | 20* | 76 | 50 | 49 |
| Croplan 822 | 25* | 50* | 50 | 42 |
| SS 842RR | 22* | 58* | 29* | 36 |
| Garst 8200YGI | 3* | 35* | 36* | 24 |
| Pioneer 31G98 | 28* | 26* | 0* | 18 |

[x]Percentages are the mean of five replicates. Number of *P. minor* per pot for Pioneer 3223 was 8,942 and 12,312 in Trial 1 and 2, respectively.
[y]Mean of three trials. Hybrids were initially evaluated in one of three experiments before being re-evaluated in Experiment 4.
[z]Asterisks next to percentages indicate a difference ($P \leq 0.05$) from the susceptible control Pioneer 3223.

3223 (Table 3). One other hybrid (Croplan 822) had significantly lower reproduction than Pioneer 3223 in Trial 1, but only numerically lower reproduction in Trial 2. Four hybrids that appeared resistant to *P. minor* in the initial experiments did not have consistently lower reproduction than Pioneer 3223 in Experiment 4. Reproduction of *P. minor* on Garst 8222IT was similar to Pioneer 3223 in all experiments.

In Experiment 4, root weights differed between trials ($P = 0.002$) and among hybrids ($P = 0.02$). The differences among hybrids in root weight was not consistent between trials ($P = 0.02$). In Trial 1, root weights were greatest in the three Garst hybrids 8200YGI, 8288, and 8222IT (86 g to 90 g), and least in Croplan 822, Southern States 842RR, and Pioneer 3223 (54 g to 58 g). In Trial 2, root weights were greatest in Southern States 842RR, Dekalb DK69-70, and Croplan 851 (71 g to 75 g), and least in Pioneer 32D99, Croplan 822, and Pioneer 31G98 (44 g to 54 g). Among hybrids, there did not appear to be any relationship between root weight and reproduction of *P. minor*.

In Experiments 5 and 6, many of the public inbred lines had numerically more *P. minor* per pot than Pioneer 3223, and two inbred lines (Va35 and Tx807) in Experiment 6 had significantly greater ($P \leq 0.05$) numbers than the control (Fig. 2). Ten inbred lines were re-evaluated for nematode reproduction in Experiment 7. Reproduction of *P. minor* differed between the two trials in Experiment 7 ($P < 0.0001$) and among the hybrids ($P < 0.0001$). The trends in reproduction among the hybrids were not consistent between the two trials ($P < 0.0001$); therefore, the trials are presented separately (Table 4). No inbred lines showed any resistance to *P. minor* relative to Pioneer 3223. However, some appeared to be more susceptible than the hybrid control in Trial 1. Va35 was the only

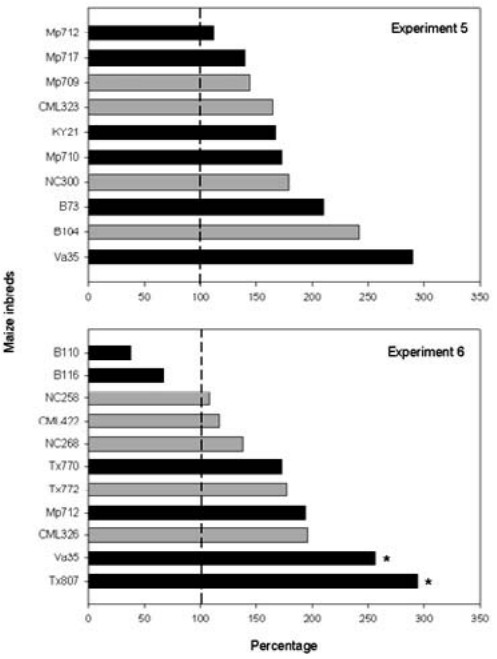


Fig. 2. Reproduction of *Paratrichodorus minor* on public maize inbred lines as a percentage of reproduction on the susceptible hybrid Pioneer 3223. The dashed line represents the reproduction on Pioneer 3223 which was 6,221 (nematodes per pot) and 6,739 in Experiments 5 and 6, respectively. Asterisks above bars indicate a difference ($P \leq 0.05$) from the control. The dark bars are inbred lines that were re-evaluated for nematode reproduction in Experiment 7.

inbred line that was consistently more susceptible to *P. minor* than Pioneer 3223. Reproduction on Va35 was 2- to 3-fold greater than on the hybrid control. Numbers of *P. minor* on the other inbred lines did not consistently differ from Va35.

In Experiment 7, root weights differed between trials ($P < 0.0001$) and among inbred lines ($P < 0.0001$). The differences among inbred lines in root weight was not consistent between trials ($P = 0.02$). In both trials, Pioneer 3223 tended to have a larger root system (75 g to 90 g) than many of the inbreds. Among the inbreds in Trial 1, root weights were greatest in B110, KY21, Tx770, and Tx807 (33 g to 41 g), and least in B116, Mp712, and Mp717 (11 g to 13 g). In Trial 2, root weights were greatest in KY21, Tx770, and Tx807 (75 g to 100 g), and least in B116, Mp710, and Mp717 (22 g to 32 g). There did not appear to be any relationship between root weight and reproduction of *P. minor* among the inbred lines.

When various inbred-hybrid combinations were compared in Experiment 8, B73 × NC246 and B116 × Mp717 appeared to be more resistant than one or both of their parents in Trial 1; however, in Trial 2, nematode reproduction was similar or greater on the hybrid than on the inbreds (Table 5). None of the inbred lines were consistently more resistant than Va35 and none of the hybrids were consistently more resistant or more susceptible than Pioneer 3223.

*Concentration of Benzoxazinones in Maize Roots*

When plant tissue is damaged, glucosides of DIMBOA and DIBOA are enzymatically hydrolyzed to the more toxic aglycones (Friebe, 2001). The benzoxazinones present in the maize roots were the MBOA aglycone (which is in the DIMBOA pathway), and glucosides of $DIM_2BOA$ (a dimethoxylated analogue of the DIMBOA) and DIMBOA, which co-eluted and could not be separately quantified. We found no DIBOA or DIMBOA aglycones. Concentrations of total benzoxazinones differed ($P = 0.005$) among maize hybrids. Pioneer 3223 contained the greatest concentration of benzoxazinones (477.6 µg/g fresh root weight) whereas the inbred Va35 contained the lowest concentration (215.6 µg/g); however, there was no relationship between concentrations of these compounds and nematode reproduction (Table 6).

## DISCUSSION

Reproduction of *P. minor* was quite variable across experiments; the reproductive factor (Pf/Pi) on Pioneer 3223 ranged

Table 4. Reproduction of *Paratrichodorus minor* on public maize inbred lines.

| Inbred | Percentage of Pioneer 3223[x] | | | |
|---|---|---|---|---|
| | Exp. 5 & 6 | Exp. 7, Trial 1 | Exp. 7, Trial 2 | Mean[y] |
| B110 | 38 | 105 | 108 | 84 |
| B116 | 67 | 161 | 96 | 108 |
| Mp 712 | 153 | 97 | 112 | 121 |
| KY21 | 167 | 184* | 36 | 129 |
| B73 | 210 | 142 | 80 | 144 |
| Tx770 | 173 | 213* | 153 | 180 |
| Tx807 | 294*[z] | 153 | 104 | 184 |
| Mp710 | 173 | 275* | 204 | 217 |
| Va35 | 273* | 200* | 200* | 224 |
| Mp717 | 174 | 453* | 48 | 225 |

[x]Percentages are the mean of five replicates. Number of *P. minor* per pot for Pioneer 3223 was 7,128 and 3,240 in Trial 1 and 2, respectively.

[y]Mean of three trials. Hybrids were initially evaluated in one of two experiments before being re-evaluated in Experiment 7.

[z]Asterisks next to percentages indicate a difference ($P \leq 0.05$) from the susceptible control Pioneer 3223.

from 6.5 to 32.4. Nevertheless, we were able to demonstrate repeatable differences in nematode reproduction in some commercial hybrids. Four hybrids (Croplan 822RR2/Bt, SS 842RR, Garst 8200YGI, and Pioneer 31G98) were moderately resistant to *P. minor*. Nematode reproduction on these four hybrids averaged less than 50% the reproduction on Pioneer 3223. Size of the root system was not responsible for this difference in reproduction. There also did not appear to be a relationship between resistance to *P. minor* and the presence of transgenes for glyphosate resistance (Roundup Ready ®) or *Bacillus thuringiensis* (*Bt*) toxin. Three of the four hybrids contained genes for glyphosate resistance; however, Pioneer 31G98, the most resistant hybrid to *P. minor*, does not contain any transgenes. Moreover, several hybrids with *Bt* or glyphosate resistance genes were as susceptible as Pioneer 3223. Resistance in commercial hybrids to *P. minor* may be the result of indirect selection based on crop performance in field sites infested with the nematode or to unintentional fixation of alleles conferring resistance in the parental inbreds.

None of the public inbred lines tested showed any resistance to *P. minor* relative to Pioneer 3223. Three of these inbred lines, Mp709, Mp710, and Mp712, are resistant to *Meloidogyne incognita* (Williams and Windham, 1998). The inbreds were derived from the open pollinated varieties Old Raccoon (Mp709 and Mp710) and Tebeau (Mp712) which express resistance through delayed nematode development (Windham and Williams, 1994). Delayed nematode development in resistant maize inbreds and varieties may be due to antibiosis from toxins produced constitutively or induced by nematode infection.

Xie *et al.* (1992) demonstrated an inverse relationship between survivorship of western corn rootworm larvae (*Diabrotica*

Table 5. Mean number of *Paratrichodorus minor* per pot containing maize inbreds and hybrids.

| Hybrid (inbred 1 × inbred 2) | Inbred 1 | Inbred 2 | Hybrid |
|---|---|---|---|
| | | Trial 1 | |
| Pioneer 3223 (control) | — | — | 7,128 |
| B73 × KY21 | 10,109[y] | 13,090 | 10,368 |
| B73 × Mp710 | 10,109 | 19,570 | 12,830 |
| B73 × Mp712 | 10,109 | 6,912 | 4,698 |
| B73 × NC246 | 10,109 a | 19,764 a[z] | 3,240 b |
| B116 × Mp717 | 11,502 b | 32,270* a | 13,446 b |
| KY21 × GT112 | 13,090 | — | 4,795 |
| Va35 (control) | 14,256 | 14,256 | |
| | | Trial 2 | |
| Pioneer 3223 (control) | — | — | 3,240 |
| B73 × KY21 | 2,592* | 1,166* | 1,555 |
| B73 × Mp710 | 2,592 | 6,610 | 4,277 |
| B73 × Mp712 | 2,592 | 3,629 | 389* |
| B73 × NC246 | 2,592 | 3,758 | 2,203 |
| B116 × Mp717 | 3,110 ab | 1,555* b | 5,702 a |
| KY21 × GT112 | 1,166* | 1,166* | 1,166 |
| Va35 (control) | 6,480 | 6,480 | |

[y]Numbers represent the mean of five replicates.
[z]In a row, different lower case letters indicate differences ($P \leq 0.05$) among inbreds and hybrids. In a column, asterisks next to means indicate a difference ($P \leq 0.05$) from the susceptible control (Pioneer 3223 for hybrids and Va35 for inbred lines).

*virgifera virgifera*) and root concentrations of the benzoxazinone DIMBOA. In the present study, we found significant differences in concentrations of benzoxazinones among the maize hybrids; however, we were unable to identify any relationship between concentration and resistance to *P. minor*. Similarly, infection of maize roots by *Pratylenchus zeae* was not affected by DIMBOA concentrations in root tissue (Friebe, 2001).

Because numbers of *P. minor* tended to be greater in the inbreds and lesser in the hybrids relative to Pioneer 3223, we hypothesized that resistance could be related to heterosis in the hybrids. However, we found no convincing evidence that hybrids were more resistant than either one or both of the inbred parental lines. The public inbred lines and hybrids made from them were similar to Pioneer 3223 in their level of susceptibility to *P. minor*. It is possible that we did not observe heterosis in the hybrids because the public inbred lines we used as parents did not contain resistance genes. The genetics of resistance to *P. minor* in maize should be investigated further.

We demonstrated a moderate level of resistance in commercial maize hybrids to the stubby-root nematode *P. minor*. How-

Table 6. Concentrations of benzoxazinones in roots of maize hybrids and one inbred (Va35) and relative reproduction of *Paratrichodorus minor*.

| Maize hybrid or inbred | $DIM_2BOA$ and DIMBOA glucosides | MBOA | Total benzoxazinones[x] | Nematode reproduction (%)[y] |
|---|---|---|---|---|
| | μg/g fresh root weight | | | |
| Pioneer 3223 | 195.4 a[z] | 282.3 ab | 477.6 a | 100 |
| Garst 8222IT | 92.1 bcd | 285.2 a | 377.2 b | 109 |
| Garst 8288 | 110.8 bcd | 257.7 abc | 368.4 b | 57 |
| Garst 8200YGI | 91.3 bcd | 264.9 ab | 356.2 bc | 24 |
| Croplan 822 | 136.0 b | 215.9 abc | 352.0 bc | 42 |
| DK69-70 | 86.8 cd | 264.0 ab | 350.8 bc | 87 |
| Pioneer 31G98 | 100.4 bcd | 245.1 abc | 345.6 bc | 18 |
| SS 842RR | 120.6 bc | 212.0 bc | 332.6 bc | 36 |
| Croplan 851 | 93.7 bcd | 213.0 bc | 307.0 bcd | 72 |
| Pioneer 30F34 | 75.2 cd | 191.4 c | 266.6 cd | 49 |
| Pioneer 32D99 | 73.1 de | 192.0 c | 265.5 cd | 83 |
| Va35 | 28.1 e | 187.5 c | 215.6 d | 224 |

[x]Sum of $DIM_2BOA$ and DIMBOA glucosides, and MBOA aglycone.
[y]Percentage reproduction of *P. minor* relative to Pioneer 3223. Percentages are the mean of three trials.
[z]Means in a column with the same letter are not different ($P \geq 0.05$).

ever, inbred sources of this resistance could not be identified in the current study because the inbred parents of the commercial hybrids were not available for testing. It seems likely that the resistance we observed in the commercial hybrids was derived from one or both of the inbred parents. Further research should be done to identify inbred lines with resistance to *P. minor*. Additionally, discovery of chemical or genetic markers for resistance would be useful for screening inbred lines and hybrids.

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