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M. P. da Silva
Iowa State University

G. L. Tylka
Iowa State University, gltylka@iastate.edu

G. P. Munkvold
Iowa State University, munkvold@iastate.edu

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M. P. da Silva, G. L. Tylka, and **G. P. Munkvold,** Department of Plant Pathology and Microbiology, Iowa State University, Ames 50011

Abstract

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Seedling diseases of maize are caused by a complex of organisms, including fungi in the genus *Fusarium*. Root-lesion nematodes (*Pratylenchus* spp.) are common in fields where maize is grown, and they are known to interact with *Fusarium* spp. in several crops. The objectives of this study were to assess the impacts of seed treatment combinations on maize seedlings coinfected with *Pratylenchus penetrans* and two *Fusarium* spp. that cause seedling disease symptoms (*Fusarium graminearum* and *F. verticillioides*) and to determine whether there were interactions between *P. penetrans* and the *Fusarium* spp. Growth-chamber experiments were conducted with fungicide- or nematicide-treated or untreated maize seed planted in a sand-soil mixture infested with inoculum of either *F. graminearum* or *F. verticillioides*. A suspension of 4,000 *P. penetrans* (mixed stages) was added to the pots at the time of planting. After 30 days, shoot length and fresh and dry shoot and root weights were determined. Total root length and fine root length, root volume, numbers of root tips and forks, and root surface area were measured through analysis of digital images of the root systems. After 42 days, *P. penetrans* nematodes were extracted and quantified from roots and soil. There were significant effects of the treatments on root health with interactions between *Fusarium* spp. and *P. penetrans*. *F. graminearum* caused the greatest reductions in root and shoot growth, and interactions with *P. penetrans* were more evident for *F. verticillioides* than for *F. graminearum*. Image analysis of root system architecture showed that seed treatment significantly improved root system characteristics. Seed treatments containing the nematicide abamectin in combination with fungicides reduced root infection by *P. penetrans* and provided the healthiest root system when under attack by the *Fusarium*–*Pratylenchus* complex.

Pratylenchus penetrans (Cobb) Filipjev & Schuurmans Stekhoven (root-lesion nematode) is one of the most common *Pratylenchus* spp., with a wide range of hosts and a cosmopolitan distribution throughout temperate regions (Corbett 1973; Loof 1991; Mai et al. 1977). This nematode is one of the most common species found on maize (*Zea mays* L.) in the United States (Norton 1984) and parts of Canada (Potter and Townshend 1973). The mechanism of pathogenesis of *P. penetrans* includes direct feeding damage to roots and interactions with other organisms in disease complexes, such as those involving fungi (Endo 1975). *P. penetrans* feeding can result in predisposition to other pathogens by wounding of the roots. Nematode–fungus interactions can result in additive or synergistic effects on disease development and impact (Back et al. 2002).

Maize seed and seedlings are susceptible to infection by several *Fusarium* spp.; *Fusarium graminearum* Schwabe and *F. verticillioides* (Sacc.) Nirenberg are among the most important species that can cause seedling diseases (Munkvold and O'Mara 2002). The symptoms caused by these pathogens are very similar: failure to emerge, wilting, chlorosis or yellowing, root rot and poor root development, slow growth and stunting, and postemergence damping-off. Seedling diseases cause losses by reducing plant populations and delaying the growth and development of surviving plants. In severe cases, seedling diseases can reduce plant population to the level that replanting is necessary (Robertson and Munkvold 2009; Stack 2000; Vincelli 2008).

The first report of an interaction between a plant-parasitic nematode and a soilborne plant-pathogenic fungus was published in 1892 (Atkinson 1892); Fusarium wilt of cotton (caused by *F. oxysporum* f. sp. *vasinfectum*) was more severe in soil coinfested with *Meloidogyne* spp. Further evidence for the interaction between *Fusarium* spp. and *Meloidogyne* spp. in cotton was later provided during field experiments, in which soil was treated with ethylene dibromide or 1,3 dichloropropene (Newson and Martin 1953; Smith 1948). It also has been shown that *Pratylenchus* spp. appear to be the dominant nematodes involved in synergistic interactions with Verticillium wilt fungi (Burpee and Bloom 1978; Huan et al. 1988; Martin et al. 1982; Mountain and McKeen 1962; Olthof and Reynes 1969; Rowe and Powelson 2002; Rowe et al. 1985).

Soil nematicides can be used to control plant-parasitic nematodes in maize, significantly reducing soil population densities. For example, applications of 1,3-D and carbofuran combined resulted in good control of *P. hexincisus* (Norton and Hinz 1976). However, use of soil-applied nematicides is often cost prohibitive, and increasing concerns about the environment, food safety, and public health has resulted in the gradual phasing out of many soil-applied nematicides (McKenry et al. 1994; United Nations Environment Programme 1992), leading to a need for alternative management tactics such as seed treatment.

Nematicides used as seed treatments are more cost-efficient and environmentally friendly tools for nematode management compared with soil applications of nematicides. For example, with the advent of products such as Avicta (abamectin; Syngenta Crop Protection, Inc.), N-Hibit (harpin protein; Plant Health Care, Inc.), Aeris (thiodicarb; Bayer Crop Science, Inc.), and Votivo bionematicide (*Bacillus firmus*; Bayer Crop Science, Inc.) as seed treatments used in cotton, soybean, and maize, more options are available to control plant-parasitic nematodes using less chemical input than was necessary with soil applications of nematicides. Abamectin proved to be very effective in reducing early infection by *Pratylenchus* spp. and *Heterodera schachtii* in maize and sugar beet roots, respectively, and also gall formation by *Meloidogyne* spp. in cotton (Cabrera et al. 2009). In fact, this study showed that abamectin at 1.0 mg of active ingredient (a.i.) seed^{-1} reduced penetration of maize roots by *P. zeae* by more than 80% during the first 14 days after nematodes were added to the soil. For this reason, assessment of the effects of these products on nematode–fungus interactions in maize is needed. Nematicidal and nematode-protectant seed treatments also provide new research tools to facilitate better understanding of the mechanisms of nematode–fungus interactions. The objectives of this study were to (i) assess the impact of seed treatment combinations (including abamectin-fungicide combinations) on maize seedlings coinfected with *P. penetrans* and *F. graminearum* or *F. verticillioides* and (ii) determine whether there were interactions between *P. penetrans* and the *Fusarium* spp., especially with respect to effects on root system growth and development.

Corresponding author: G. P. Munkvold. munkvold@iastate.edu

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Materials and Methods

Experimental design. Separate experiments were conducted with either *F. verticillioides* or *F. graminearum*. Each experiment was conducted twice in a growth chamber at Iowa State University, Ames. A full factorial experimental design was used. Experimental factors were seed treatment (eight treatments), *P. penetrans* treatment (infested or not), and fungal treatment (infested or not). Seed of maize NK Brand hybrid N40T-GT (Syngenta Seeds) was commercially treated by the manufacturer with seven different combinations of active ingredients, and a nontreated control was included. All treatments included a base combination of the fungicides fludioxonil, mefenoxam, and azoxystrobin (FMA), and some treatments included an additional fungicide (thiabendazole), insecticide (thiamethoxam), or nematicide (abamectin) (Table 1). Seed treatments were manufacturer (Syngenta Crop Protection) formulations applied at the recommended rates for commercial use. The 32 treatment combinations were arranged in a growth chamber as a randomized complete block design with six replicates. Plant were grown in 164-ml cones (Ray Leach Cone-tainers, model SC10; Stuewe and Sons, Inc.). Each cone was an experimental unit. Plants were maintained in a growth chamber under light supplied by cool-white fluorescent and incandescent lamps with a photoperiod of 14 h. Relative humidity was maintained at 99% and temperature was 22 ± 0.1°C. The plants were watered once a day using a watering can (20 ml/plant) and fertilized once a week, using Peters Excell water-soluble fertilizer (15-5-15; Everris U.S., Inc.). A small piece of paper towel in the bottom of each cone partially reduced drainage.

Fungal and nematode infestation. *Fusarium* isolates ISUA66A (*F. graminearum*) and ISU93048 (*F. verticillioides*), isolated from kernels of maize grown in Iowa, were used in the experiments. Inoculum of *Fusarium* isolates was prepared following the procedure described by Munkvold and O'Mara (2002), modified from that of Desjardins et al. (1995). A mixture of sand (1,900 ml), corn meal (380 ml), and water (110 ml) was autoclaved in bags for 1 h at 121°C on two consecutive days. Spore suspensions (10^6 conidia ml^{-1}) of the *Fusarium* isolates were prepared from cultures on carnation leaf agar (Leslie and Summerell 2006). A spore suspension (2 ml) of one of the *Fusarium* isolates was injected into each bag. The bags were then incubated in the dark at ambient temperature (20 to 24°C) for 6 days, with mixing every day. Autoclaved sand-soil (1 part soil to 2 parts sand) was mixed with the fungal inoculum. The proportion was 30% of inoculum and 70% by volume of the sand-soil mixture. Cones were filled with the mixture and one maize seed was placed in each cone. *P. penetrans,* provided by Dr. A. E. MacGuidwin (University of Wisconsin, Madison), was cultured monoxenically (Layne and MacGuidwin 1994) on excised maize roots in Gamborg's B-5 medium with vitamins and without cytokinins or auxin (Gamborg et al. 1976). Agar surfaces of 3-month-old cultures were rinsed with sterile distilled water to collect nematodes (Layne and MacGuidwin 1994). The nematode inoculum was prepared in a water suspension in a total volume of 50 ml, which was then diluted to achieve a density of 2,000 nematodes ml^{-1} determined by counting using a nematode counting slide under a dissecting microscope. Nematodes were added to the cones by injecting a 2-ml nematode suspension (equivalent to 4,000 nematodes) to a depth of approximately 2.5 cm using a microliter pipette at the time of planting (Saeed et al. 1999).

Data collection and analysis. Seedling emergence was recorded daily from the day the first plant emerged until 30 days after planting. Four replicates were harvested 30 days after planting. Plants were removed from the cones, soil adhering to the roots was shaken free, and the roots were washed thoroughly. Shoot lengths (flag leaf) and fresh shoot and root weights were measured. Shoots and roots were oven dried at 110°C for 24 h and weighed.

To assess root morphology for each treatment, roots were scanned and images analyzed using the software WinRhizo 2008a (Regent Instruments Inc.). Intact washed roots were spread in a transparent tray in order to avoid overlapping during the scanning process. Image recording was performed at a resolution of 600 dots per inch using a 24-bit color mode, and images were saved as TIFF files. All other scanner settings (such as dust removal) were turned off. A Dell Precision T3500 desktop computer (Dell Inc.) was used to drive the Epson Perfection V700 Photo-Dual Lens System scanner (Seiko Epson Corp.). Root morphology was examined and the following measurements were recorded: total root length (centimeters), total surface area (square centimeters), total volume (cubic centimeters), number of tips, number of forks, and length (centimeters) of fine roots (<0.5 mm in diameter).

Two replicates per treatment were harvested after 6 weeks in order to extract *P. penetrans* from soil and roots. A 100 cm^3 soil sample was collected after the soil was removed from the cones, thoroughly mixed, and *P. penetrans* were extracted using the centrifugal flotation technique (Jenkins 1964). Roots were cut into small pieces (1 cm) long, mixed and incubated in Baermann funnels for 2 days (De Waele and Elsen 2002). After nematodes were collected, the roots were dried at 100°C for 2 days and weighed. The number of *P. penetrans* (juveniles and adults) that had emerged from the roots was counted using an inverted microscope and a nematode-counting slide. The total number of nematodes present per pot was calculated based on soil volume and root weight. Also, the number of *P. penetrans* nematodes per gram of dry root weight was determined.

Data were analyzed by analysis of variance (ANOVA) for a completely randomized block design (SAS Inc.). Least significant differences ($\alpha \leq 0.05$) for comparing treatment means also were calculated according to the GLM procedure of SAS. In order to meet ANOVA assumptions of normality and equal variances, ANOVA were performed on transformed ($\log_{10}$) data, except for nematode population data, which were not transformed. Analysis of all main effects and interactions was conducted using all treatment combinations. Because of significant seed treatment–pathogen infestation interactions, seed treatment effects also were tested for treatments infested with *Fusarium* spp. and *P. penetrans* only, excluding the noninfested treatment combinations. Data from the two runs of each experiment were combined for analysis.

Results

***F. graminearum* experiments.** Fungal infestation strongly affected all the variables measured, whereas nematode infestation affected only

Table 1. Seed treatments used in the experiments[z]

Treatment	Active ingredients	Chemical group	Formulation (%)	Brand name	Rate
1	Fludioxonil	Phenylpyrrole	40.3	Maxim	2.5 g/100 kg
	Mefenoxam	Phenylamide	1.1	Apron XL	2 g/100 kg
	Azoxystrobin	Strobilurin	9.6	Dynasty	1 g/100 kg
2	FMA + thiabendazole	Benzimidazole	42.3	MaximQuattro	20 g/100 kg
3	FMA + thiamethoxam	Neonicotinoid	47.6	Cruiser	0.25 mg/seed
4	FMA + thiamethoxam + thiabendazole	…	47.6	CruiserMaxx	…
5	FMA + abamectin	Avermectin	46.3	Avicta	0.25 mg/seed
6	FMA + abamectin + thiabendazole	…	12.4	…	…
7	FMA + abamectin + thiamethoxam + thiabendazole	…	12.4	…	…
8	Untreated	…	0	…	…

[z] Treatment 1 is the commercial standard and consists of fludioxonil + mefenoxam + azoxystrobin (FMA), and treatments 2 through 7 include FMA at the same rates as treatment 1.

shoot dry and root dry weights as a main effect. Also, there were significant interactions between *F. graminearum* and *P. penetrans* affecting root dry weights and fine roots (Table 2). Seed treatment significantly affected all the root variables except weight but did not have a main effect on shoot variables. There were strong interactions between seed treatment and fungal infestation effects for all the variables measured, reflecting the fact that the effects of most seed treatments occurred only when the fungus was present. There were significant interactions between seed treatment and nematode infestation affecting shoot length, shoot fresh weight, and root fresh weight. There were no significant three-way interactions between seed treatment, fungal infestation, and nematode infestation for any of the measured variables. There were significant effects of seed treatment on the numbers of nematodes extracted from soil ($P < 0.0001$) and from the roots ($P = 0.004$); treatments including the nematicide abamectin were the only ones differing significantly from the untreated control (Table 3).

Seed treatments (which all contained fungicides) were very effective for improving seedling health in *F. graminearum*-infested treatments; all seed treatments significantly improved all variables compared with the untreated control, except for fine root length. Values for measurements of seedling morphology were often increased more than twofold by seed treatments in comparison with the untreated control. Treatments 6 (FMA + abamectin + thiabendazole) and 7 (FMA + abamectin + thiamethoxam + thiabendazole) often resulted in the highest means but, in many cases, the means were not significantly different from the other seed treatment combinations (Table 4). In *P. penetrans*-infested treatments, there were significant seed treatment effects for all variables except fine root length but not all the seed treatments differed from the untreated control (Table 4); treatments including abamectin most often differed from the control. Treatment 6 (FMA + abamectin + thiabendazole) was significantly different from the untreated control for all treatments except fine root length. Seed treatments did not affect any of the measured variables in the noninfested control treatments. *P. penetrans* was not detected in noninfested controls.

***F. verticillioides* experiments.** Effects of this fungus were weaker than those of *F. graminearum.* As a main effect, *F. verticillioides* infestation significantly affected root length, root volume, number of tips, number of forks, root surface area, and number of fine roots. At the same time, nematode infestation significantly affected root length, number of tips, number of forks, root surface area, number of fine roots, and root volume (Table 2). There were significant interactions between seed treatment and fungal infestation for all the variables except dry weights and shoot length. There were significant interactions between seed treatment and nematode infestation affecting shoot length and fresh weight, root length, forks, and fine roots. Compared with the *F. graminearum* experiments, there were many more significant interactions between *F. verticillioides* infestation and nematode infestation; these occurred for root length, number of tips, number of forks, root surface area, and number of fine roots (Table 2). There was a significant effect of seed treatment ($P = 0.0005$) on the numbers of *P. penetrans* extracted from soil. There was a significant main effect of seed treatment ($P = 0.003$) and also a significant interaction between seed treatment and fungal infestation ($P = 0.01$) affecting the numbers of nematodes extracted from the roots; treatments including the nematicide abamectin were the only ones differing significantly from the untreated control (Table 3). In *F. verticillioides*-infested treatments, there were significant main effects of seed treatment for all the variables except shoot length but treatment combinations rarely differed significantly from each other and, in many cases, only treatment 7 (FMA + thiabendazole + thiamethoxam + abamectin) differed from the untreated control (Table 5). In *P. penetrans*-inoculated treatments, there were fewer significant seed treatment effects (shoot length, shoot fresh weight, root length, number of forks, and length of fine roots) and the different treatment combinations rarely differed from each other (Table 5). Seed treatments did not affect any of the measured variables in the noninfested control treatments. *P. penetrans* was not detected in noninfested controls.

Discussion

Infestation of the potting medium with *Fusarium* spp. or *P. penetrans* had detrimental effects on most of the seedling health and root morphology variables measured in this study. Fungal infestation, particularly with *F. graminearum*, had a much larger effect than

Table 2. *P* values for main and interactive effects of seed treatments and pathogen inoculation on seedling health variables for *Fusarium graminearum* and *F. verticillioides* experiments[y]

		Effects[z]						
Experiment	**Variable**	**ST**	**F**	**N**	**ST × F**	**ST × N**	**F × N**	**ST × F × N**
F. graminearum	Shoot length (cm)	ns	<0.0001	ns	<0.0001	0.02	ns	ns
	Shoot fresh (g)	ns	<0.0001	ns	<0.0001	0.01	ns	ns
	Shoot dry (g)	ns	<0.0001	0.003	<0.0001	ns	ns	ns
	Root fresh (g)	ns	<0.0001	ns	<0.0001	0.008	ns	ns
	Root dry (g)	ns	<0.0001	0.004	<0.0001	ns	0.009	ns
	Root length (cm)	0.0002	<0.0001	ns	0.0009	ns	ns	ns
	Root volume (cm^3)	0.01	<0.0001	ns	<0.0001	ns	ns	ns
	Tips	0.001	<0.0001	ns	0.01	ns	ns	ns
	Forks	0.0003	<0.0001	ns	0.001	ns	ns	ns
	Surface area (cm^2)	<0.0001	<0.0001	ns	<0.0001	ns	ns	ns
	Fine roots (cm)	0.002	<0.0001	ns	<0.0001	ns	0.04	ns
F. verticillioides	Shoot length (cm)	ns	ns	ns	ns	0.005	ns	ns
	Shoot fresh (g)	ns	ns	ns	0.009	0.004	ns	ns
	Shoot dry (g)	ns	ns	ns	ns	ns	ns	ns
	Root fresh (g)	ns	ns	ns	0.03	ns	ns	ns
	Root dry (g)	ns	ns	ns	ns	ns	ns	ns
	Root length (cm)	ns	<0.0001	<0.0001	0.001	0.03	0.004	ns
	Root volume (cm^3)	ns	<0.0001	0.001	0.01	ns	ns	ns
	Tips	ns	<0.0001	<0.0001	0.004	ns	0.004	ns
	Forks	ns	<0.0001	<0.0001	0.003	0.009	0.005	ns
	Surface area (cm^2)	ns	<0.0001	<0.0001	0.001	ns	0.009	ns
	Fine roots (cm)	ns	<0.0001	<0.0001	0.001	0.04	0.001	ns

[y] Analysis of variance was conducted with $\log_{10}$ transformed data except for disease root length (%) and disease root volume (%), which were not transformed. Separate experiments were conducted with *F. graminearum* and *F. verticillioides*. Data were combined for two runs of each experiment; ns = not significant ($P > 0.05$).

[z] Abbreviations: ST = seed treatment, F = fungus, and N = nematode.

Table 3. Mean number of *Pratylenchus penetrans* recovered from 100 cm^3 of soil and the maize roots therein and from 1 g (dry weight) of maize roots from treatments infested with the nematodes and with or without *Fusarium* spp. infestation[y]

Experiment, treatment	Active ingredients[z]	Nematodes/100 cm^3 of soil	Nematodes/g of root
Fusarium graminearum			
1	FMA	57.5 b	17.5 a
2	FMA + thiabendazole	60.8 b	13.3 a
3	FMA + thiamethoxam	49.3 b	12.0 a
4	FMA + thiamethoxam + thiabendazole	62.3 b	10.0 ab
5	FMA + abamectin	171.8 a	1.8 c
6	FMA + abamectin + thiabendazole	156.5 a	0.0 c
7	FMA + abamectin + thiamethoxam + thiabendazole	205.5 a	0.0 c
8	Untreated	81.5 b	9.5 ab
F. verticillioides			
1	FMA	47.7 c	10.0 abc
2	FMA + thiabendazole	49.7 c	16.0 ab
3	FMA + thiamethoxam	77.7 c	12.0 ab
4	FMA + thiamethoxam + thiabendazole	42.2 bc	18.7 a
5	FMA + abamectin	148.7 a	0.0 c
6	FMA + abamectin + thiabendazole	110.2 a	0.2 c
7	FMA + abamectin + thiamethoxam + thiabendazole	140.2 a	0.0 c
8	Untreated	65.5 bc	7.5 bc

[y] Values are means of eight plants (two replications per treatment combination × two runs of the experiment).Values within a column and experiment followed by the same letter are not significantly different according to Fisher's least significant difference ($\alpha = 0.05$).

[z] FMA = fludioxonil + mefenoxam + azoxystrobin = seed treatment 1.

P. penetrans infestation for most of the variables. There were large differences in root morphology between *F. graminearum*-infested treatments and *P. penetrans*-infested treatments; for example, overall root length was about 80% lower (Table 4) and fine root length was about 90% lower (data not shown) for *F. graminearum*-infested treatments. This reflects the severe effect of *F. graminearum* on root health; *P. penetrans* infestation alone, however, had a relatively small effect on root characteristics that we measured. There were significant interactions between *F. graminearum* and *P. penetrans* affecting root dry weight and the number of fine roots but the overwhelming effects of *F. graminearum* on the root variables may have detracted from the ability of the nematode to colonize roots and, therefore, limited the possibility of other interactions between the pathogens. In contrast, there were significant interactions between *F. verticillioides* and *P. penetrans* for several root health variables, and this may have occurred because *F. verticillioides* had a less drastic effect on the roots than *F. graminearum.*

There were several noticeable contrasts between the two *Fusarium* spp. regarding the effects of seed treatment, fungal infestation, nematode infestation, and fungus–nematode interactions (Table 2). For instance, there were greater effects of nematode infestation and fungus–nematode interactions in experiments with *F. verticillioides* than in experiments with *F. graminearum*. The *F. verticillioides* isolate used in the study was less aggressive than the *F. graminearum* isolate but displayed more evidence of significant interactions with *P. penetrans* for several variables. This observation suggests that the more aggressive *F. graminearum* isolate was better able to infect roots without the aid of the nematode but *F. verticillioides* was less effective without the aid of the nematode.

These results support previous research on synergistic interactions with *F. verticillioides* and plant-parasitic nematodes. Palmer and MacDonald (1974) and Palmer et al. (1967) reported a synergistic interaction between *P. scribneri* and *F. verticillioides* affecting fresh weight of maize seedlings in the presence of both pathogens. Moreover, Jordaan et al. (1987) reported that a combination of *P. brachyurus* and *P. zeae* can interact with the root-rot fungus *F. moniliforme* (syn. *F. verticillioides*) on maize, and this interaction

Table 4. Shoot and root measurements for maize seedlings grown from seed treated with different seed treatment products and grown in a sand/soil mixture infested with *Fusarium graminearum* inoculum or *Pratylenchus penetrans* adults and juveniles[x]

Infestation, treatment[y]	Active ingredients[z]	Shoot length (cm)	Shoot fresh weight (g)	Root length (cm)	Root fresh weight (g)	Root volume (cm^3)	Root tips (*n*)
F. graminearum							
1	FMA	18.4 a	1.02 ab	40.9 b	1.04 a	0.58 b	68.5 ab
2	FMA + thiabendazole	19.3 a	1.13 ab	44.8 ab	1.06 a	0.58 b	64.6 bc
3	FMA + thiamethoxam	17.1 a	0.95 b	46.4 ab	0.98 a	0.67 ab	72.0 ab
4	FMA + thiamethoxam + thiabendazole	19.1 a	0.94 b	48.3 ab	0.93 a	0.62 b	75.1 ab
5	FMA + abamectin	19.5 a	0.96 ab	49.4 ab	0.91 a	0.68 ab	68.5 ab
6	FMA + abamectin + thiabendazole	19.5 a	1.31 a	53.1 a	1.17 a	0.78 a	82.4 a
7	FMA + abamectin + thiamethoxam + thiabendazole	18.3 a	1.06 ab	52.9 a	1.08 a	0.69 ab	79.1 ab
8	Untreated	10.2 b	0.35 c	28.2 c	0.33 b	0.29 c	48.3 c
P. penetrans							
1	FMA	28.9 a	2.89 b	168.9 b	2.53 abc	1.18 b	290 b
2	FMA + thiabendazole	27.4 bc	2.62 bc	186.1 ab	2.36 abc	1.39 b	301 ab
3	FMA + thiamethoxam	26.4 bc	2.63 bc	189.0 ab	2.38 bc	1.45 ab	301 ab
4	FMA + thiamethoxam + thiabendazole	28.3 b	2.61 bc	186.4 ab	2.44 bc	1.48 ab	314 ab
5	FMA + abamectin	26.8 bc	2.72 bc	192.0 ab	2.10 cd	1.51 ab	316 ab
6	FMA + abamectin + thiabendazole	33.1 a	3.67 a	225.4 a	3.11 a	1.89 a	404 a
7	FMA + abamectin + thiamethoxam + thiabendazole	28.8 b	2.89 b	208.4 ab	2.81 ab	1.56 ab	329 ab
8	Untreated	24.0 c	2.12 c	159.0 b	1.76 d	1.15 b	262 b

[x] Values are means of two experiments with eight replications each. Values within a column and infestation treatment followed by the same letter are not significantly different according to Fisher's least significant difference ($\alpha = 0.05$).

[y] *F. graminearum*-infested treatments include all those that were infested with *F. graminearum* (four replications with the fungus alone and four replications infested with both pathogens). *P. penetrans*-infested treatments include all those that were infested with *P. penetrans* (four replications with the nematode alone and four replications infested with both pathogens).

[z] FMA = fludioxonil + mefenoxam + azoxystrobin = seed treatment 1.

can cause more severe effects on plant growth than the nematodes or fungus alone. A recent preliminary report by Lunt and MacGuidwin (2014) indicated that maize seedling growth did not differ significantly between single-pathogen and coinoculated treatments when *P. penetrans* was combined with *F. verticillioides*. This observation differs from the results reported here, potentially due to the different environmental and inoculum conditions between the two studies. *Fusarium* isolates within a species can display considerable variability (Desjardins 2006); therefore, it is not clear whether our results regarding differences between *F. graminearum* and *F. verticillioides* would be consistent across multiple isolates of each species.

In treatments infested with either or both pathogens, seed treatment combinations that included abamectin or abamectin with thiabendazole resulted in the healthiest and largest root systems compared with the untreated control or to a fungicide or insecticide seed treatment without abamectin. These results are consistent with previous work in cotton (Monfort et al. 2006), in which the authors reported increased plant height in abamectin-treated plants compared with control plants. Seed treatment effects were very prominent in the *F. graminearum*-infested treatments, where all seed treatment combinations resulted in significant improvements in all seedling variables compared with the untreated control. A likely explanation is the inclusion of fludioxonil, which is highly active against most isolates of *F. graminearum* (Munkvold and O'Mara 2002), in all the treatment combinations. Conditions in our growth chamber assay may have favored the demonstration of seed treatment efficacy because of the low soil volume, the soil mix that contained a high proportion of sand, and the use of high numbers of nematodes placed near the seed. Conversely, daily watering of the sand-soil mixture can leach away seed treatment active ingredients, and we did not observe abnormally high levels of *P. penetrans* infection in the untreated control, in spite of the high numbers added to each cone. Although the magnitude of seed treatment effects in this assay may not mimic those observed in the field, the assay provided an effective measure of the relative efficacy of the various treatments.

Population densities of *P. penetrans* from soil and roots were significantly affected by seed treatment. Seed treatment combinations with abamectin had lower population densities of *P. penetrans* in the roots but higher population densities in the soil compared with treatments without abamectin (Table 3). Apparently, seed treatment combinations containing abamectin protected the maize roots, reducing nematode penetration and altering the ratio of *P. penetrans* in the soil versus in the roots. Similarly, Cabrera et al. (2009), working with efficacy of abamectin seed treatment on *P. zeae*, found that penetration of *P. zeae* was reduced more than 80% in maize at a dose of 1.0 mg a.i./seed. In the current study, *P. penetrans* population densities in roots also were significantly reduced by seed treatment combinations with abamectin, possibly resulting in higher numbers of nematodes recovered from soil. Abamectin is a broad-spectrum nematicide providing protection against a wide range of plant-parasitic nematode genera, including *Belonolaimus, Criconemella, Helicotylenchus, Hoplolaimus, Longidorus, Meloidogyne, Pratylenchus, Trichodorus, Tylenchorhynchus*, and *Xiphinema* (Lasota and Dybas 1990). However, abamectin used as a seed treatment nematicide provides early-season, not season-long, nematode protection, and the duration of protection is not specified or known. Thiabendazole has been reported to have some nematicidal activity against some species of nematode parasites that occurs in the upper digestive tract of various mammals (Kudo et al. 2008). There may be some evidence of such nematicidal activity in our study. For *P. penetrans*-infested treatments, only the seed treatment combination with both abamectin and thiabendazole was significantly different from the untreated control for several of the plant growth variables.

Emergence rate (data not shown) did not differ significantly among treatments, and all treatments were fully emerged at 20 days after planting. *P. penetrans* is not known to reduce emergence, and the temperature used in the experiments was not optimal for stand reduction by *Fusarium* spp. The temperature used in these experiments was within the optimal range for *P. penetrans* (22 to 25°C) (Castillo and Volvas 2007). *Fusarium* spp. can cause more severe symptoms in maize seed and seedlings when soil temperatures are below 13°C (Smith and White 1988), mainly because germination is greatly retarded in this temperature range. Therefore, in order to

Table 5. Shoot and root measurements for maize seedlings grown from seed treated with different seed treatment products and grown in a sand/soil mixture infested with *Fusarium verticillioides* inoculum or *Pratylenchus penetrans* adults and juveniles[x]

Infestation, treatment[y]	Active ingredients[z]	Shoot length (cm)	Shoot fresh weight (g)	Root length (cm)	Root fresh weight (g)	Root volume (cm^3)	Root tips (*n*)
F. verticillioides							
1	FMA	26.6 a	2.28 ab	94.1 ab	1.49 ab	0.44 bc	188.1 ab
2	FMA + thiabendazole	31.5 a	2.50 ab	101.2 ab	1.40 ab	0.49 bc	190.2 ab
3	FMA + thiamethoxam	28.8 a	2.46 ab	98.5 ab	1.44 ab	0.48 bc	205.4 a
4	FMA + thiamethoxam + thiabendazole	28.8 a	2.55 ab	113.1 ab	1.54 ab	0.56 ab	196.2 ab
5	FMA + abamectin	34.0 a	2.54 ab	108.3 ab	1.43 ab	0.57 ab	208.7 a
6	FMA + abamectin + thiabendazole	32.2 a	2.65 ab	130.4 a	1.62 ab	0.56 ab	249.9 a
7	FMA + abamectin + thiamethoxam + thiabendazole	32.4 a	3.11 a	124.9 a	1.78 a	0.65 a	255.3 a
8	Untreated	26.4 a	1.99 b	78.5 b	1.24 b	0.34 c	177.0 b
P. penetrans							
1	FMA	23.7 ab	1.89 b	181.8 ab	1.33 a	0.79 a	306 a
2	FMA + thiabendazole	29.9 ab	2.56 ab	189.4 ab	1.52 a	0.84 a	306 a
3	FMA + thiamethoxam	27.9 ab	2.47 ab	189.4 ab	1.49 a	0.81 a	315 a
4	FMA + thiamethoxam + thiabendazole	30.7 ab	2.42 ab	185.6 ab	1.49 a	0.85 a	331 a
5	FMA + abamectin	29.9 ab	2.71 a	197.0 ab	1.69 a	0.91 a	379 a
6	FMA + abamectin + thiabendazole	30.1 ab	2.71 a	197.0 ab	1.53 a	0.91 a	347 a
7	FMA + abamectin + thiamethoxam + thiabendazole	32.2 a	2.71 a	212.1 a	1.66 a	0.90 a	379 a
8	Untreated	19.2 b	1.84 b	168.9 b	1.31 a	0.71 a	290 a

[x] Values are means of two experiments with eight replications each. Values within a column and infestation treatment followed by the same letter are not significantly different according to Fisher's least significant difference ($\alpha = 0.05$).

[y] *F. verticillioides*-infested treatments include all those that were infested with *F. verticillioides* (four replications with the fungus alone and four replications infested with both pathogens). *P. penetrans*-infested treatments include all those that were infested with *P. penetrans* (four replications with the nematode alone and four replications infested with both pathogens).

[z] FMA = fludioxonil + mefenoxam + azoxystrobin = seed treatment 1.

fully understand *P. penetrans* interactions with seedling pathogens, these studies should be repeated under a range of temperatures.

Image analysis of root structure analysis using WinRhizo showed that seed treatments significantly improved root system characteristics such as root volume, root length, number of tips, forks, surface area, and fine root development. Some variables calculated by the software were highly correlated and, in this study, demonstrated very similar treatment effects (e.g., root volume and surface area, and root tips and forks). Fine root length (data not shown) was highly correlated with total root length and, in nearly all cases, seed treatments increased fine root length compared with the control; however, there were few differences among treatments for this variable. Image analysis facilitated more precise quantification of root health and morphology variables in order to measure pathogen and seed treatment effects on roots. WinRhizo has been reported to provide accurate measurements of root morphological parameters (Himmelbauer et al. 2004). Root morphological characteristics measured in this study, including length and surface area, are important indicators for potential uptake of water and nutrients (Himmelbauer et al. 2004; Pallant et al. 1993; Zobel et al. 2007). Root image analysis data indicated similar effects of fungal and nematode infestation but effects were more dramatic with *F. graminearum* than with *F. verticillioides*. Furthermore, there were significant seed treatment interactions with both fungal and nematode infestation, reducing root rot and enhancing root system characteristics in infested treatments. There were no significant three-way interactions, suggesting that seed treatment did not alter the nature of the *Fusarium–Pratylenchus* interaction.

The seed treatments in this study had no effect on the plant growth variables in treatment combinations that were not infested with *Fusarium* spp. or *P. penetrans* in these experiments. The absence of such effects indicates that seed treatment effects on maize seedlings were due to suppression of pathogen activity, and not direct effects on plant physiology. Although thiamethoxam seed treatment has been associated with direct physiological effects on plants (Perelló and Dal Bello 2011), we did not see evidence for that in this study; treatment 7 (with thiamethoxam) rarely differed from treatment 6 (the same combination without thiamethoxam). There were no negative effects concerning phytotoxicity, such as lower germination, stunting, or chlorosis.

To our knowledge, this study is the first to assess the interactions between *P. penetrans* and *Fusarium* spp. on maize roots in relation to the potential benefits of abamectin combined with fungicidal seed treatment. Data obtained in this study provide evidence that abamectin in combination with fungicidal seed treatments significantly improved the protection of the maize root system against *P. penetrans* infection and seedling disease symptoms. In addition, our research presents novel data regarding root system characteristics in response to fungal and nematode infestation and seed treatments, as measured by root image analysis.

The results demonstrated significant effects of seed treatments on root health with interactions between fungal pathogens and *P. penetrans*. Seed treatments showed efficacy against fungal and nematode infestations, improving most measures of seedling health compared with the untreated control, particularly seed treatment combinations that included abamectin and thiabendazole. Root structure analysis using WinRhizo was a powerful tool to demonstrate benefits of seed treatment toward improved root system characteristics such as root volume, root length, number of tips, forks, surface area, and fine roots.

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