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The roots of maize seedlings typically are attacked by a complex of organisms that includes fungal pathogens and plant-parasitic nematodes but few studies have examined the effects of these organisms in combination. *Rhizoctonia solani* can be an important component of the seedling disease complex; like other fungi, its effect on the plant may be influenced by the activity of nematodes such as the root-lesion nematode *Pratylenchus penetrans*. In this study, we assessed the impact of seed treatments, including fungicide–nematicide combinations, on maize seedlings exposed to *R. solani* and *P. penetrans* alone or in combination. In growth-chamber and greenhouse experiments, seed treated with various active ingredient combinations were planted in an autoclaved sand-soil mixture with or without inoculum of *R. solani*. In some treatments, a suspension of *P. penetrans*adults and juveniles was added to the sand-soil mixture. In the greenhouse experiments, infection by *R. solani* caused dramatic reductions in root length, volume, surface area, and numbers of root tips and root forks, whereas *P. penetrans* infestation alone reduced only shoot fresh weight. Statistical interactions between the effects of the two organisms were not significant, although fungal infestation significantly reduced the numbers of nematodes extracted from roots. Seed treatments significantly improved most root development variables, and the combination that included four fungicides, thiamethoxam, and abamectin was the best treatment for most variables. Results were similar in the growth-chamber experiments, where *R. solani* caused significant reductions in nearly all shoot and root development measurements, and seed treatment with sedaxane, alone or combined with abamectin, consistently provided the best results. *R. solani* was more damaging to seedlings than *P. penetrans*, and the combination of the two organisms did not cause more damage than *R. solani* alone. Seed-treatment active ingredients that specifically targeted *R. solani* (sedaxane) and *P. penetrans* (abamectin) had large positive effects on seedling health, causing significant improvements in root and shoot growth and development compared with untreated seedlings exposed to these pathogens.

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Seed Treatment Effects on Maize Seedlings Coinfected with *Rhizoctonia solani* and *Pratylenchus penetrans*

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Abstract

The roots of maize seedlings typically are attacked by a complex of organisms that includes fungal pathogens and plant-parasitic nematodes but few studies have examined the effects of these organisms in combination. *Rhizoctonia solani* can be an important component of the seedling disease complex; like other fungi, its effect on the plant may be influenced by the activity of nematodes such as the root-lesion nematode *Pratylenchus penetrans*. In this study, we assessed the impact of seed treatments, including fungicide–nematicide combinations, on maize seedlings exposed to *R. solani* and *P. penetrans* alone or in combination. In growth-chamber and greenhouse experiments, seed treated with various active ingredient combinations were planted in an autoclaved sand-soil mixture with or without inoculum of *R. solani.* In some treatments, a suspension of *P. penetrans* adults and juveniles was added to the sand-soil mixture. In the greenhouse experiments, infection by *R. solani* caused dramatic reductions in root length, volume, surface area, and numbers of root tips and root forks, whereas *P. penetrans* infestation alone reduced only shoot fresh weight. Statistical interactions between the effects of the two organisms were not significant, although fungal infestation significantly reduced the numbers of nematodes extracted from roots. Seed treatments significantly improved most root development variables, and the combination that included four fungicides, thiamethoxam, and abamectin was the best treatment for most variables. Results were similar in the growth-chamber experiments, where *R. solani* caused significant reductions in nearly all shoot and root development measurements, and seed treatment with sedaxane, alone or combined with abamectin, consistently provided the best results. *R. solani* was more damaging to seedlings than *P. penetrans*, and the combination of the two organisms did not cause more damage than *R. solani* alone. Seed-treatment active ingredients that specifically targeted *R. solani* (sedaxane) and *P. penetrans* (abamectin) had large positive effects on seedling health, causing significant improvements in root and shoot growth and development compared with untreated seedlings exposed to these pathogens.

Maize (*Zea mays* L.) seed and seedlings are susceptible to infection by a number of soilborne pathogens that can cause seedling diseases; and, in many cases, multiple pathogens can be recovered from diseased seedlings. Symptoms such as pre- or postemergence damping-off, wilting, chlorosis or yellowing, root rot and poor root development, slow growth, and stunting can be caused by numerous pathogens, including fungi, Oomycetes, and plant-parasitic nematodes, often in combination. Seedling diseases can reduce plant populations to the extent that replanting sometimes is necessary, and also may reduce the productivity of surviving plants (Robertson and Munkvold 2009; Stack 2000; Vincelli 2008).

Rhizoctonia solani is the most widely recognized species of *Rhizoctonia*. It can cause damping-off of young seedlings in many different crops and is a major component of seedling disease complexes in the central United States (Rizvi and Yang 1996). *R. solani* attacks all belowground plant parts, including seed, hypocotyls, and roots. *R. solani* is divided into anastomosis groups (AG) based on hyphal anastomosis and cultural characteristics (Dorrance et al. 2003; Ogoshi 1987; Sneh et al. 1991). Isolates within an AG may have analogous characteristics such as symptoms produced on a host and host preferences (Anderson 1982; Sneh et al. 1991). Maize is a host for several *R. solani* AG and subgroups. For example, Sumner and Bell (1982) reported AG 2-2 and AG-4 to be pathogenic on maize in Georgia. Similarly, Ithurrart et al. (2004) demonstrated that maize serves as a host plant for *R. solani* AG 2-2IIIB.

Fungi play an important role in the etiology of several diseases caused by a fungus–nematode complex (Powell 1971). The combination of plant-parasitic nematode and fungus often results in a synergistic interaction, wherein the damage is greater than expected from either pathogen alone or there is an additive effect of the two together. Root-rot pathogens such as *R. solani* can be involved in nematode disease complexes (Back et al. 2002; Chand et al.1985). For example, *Meloidogyne incognita* predisposes tomato and tobacco plants to subsequent infection when exposed to either *R. solani* or *Pythium ultimum* (Nava 1970). Also, based on results of Polychronopoulos et al. (1969), *Heterodera schachtii* (the beet cyst nematode) can facilitate the infection of sugar beet roots by *R. solani*.

Pratylenchus penetrans (the root-lesion nematode) is one of the most common species of plant-parasitic nematodes found on maize in the United States (Lopez Nicora et al. 2011; Norton 1984; Simon et al. 2014) and Eastern Canada (Potter and Townshend 1973). *P. penetrans* is a destructive migratory endoparasite of the root cortex. *P. penetrans* can affect the host directly or through interactions with other organisms in disease complexes such as those involving fungi, including *Fusarium* spp., *R. solani*, and *Verticillium albo-atrum* (da Silva et al. 2016; Endo 1975; Palmer and MacDonald 1974). However, *P. penetrans* interactions with *R. solani* have not been studied on maize, and more research is needed in order to better understand the importance of these interactions.

The maize seedling disease complex is usually managed through the use of seed treatments, and commercial formulations often include combinations of fungicides, an insecticide, and a nematicide. Several broad-spectrum fungicides are commonly used, including azoxystrobin (Fungicide Resistance Action Committee [FRAC] code 11), fludioxonil (FRAC code 12), and thiabendazole (FRAC code 1) (Munkvold et al. 2014). These three active ingredients all have varying degrees of activity against *R. solani* (Brantner et al. 2012; Hamada et al. 2011; Leach and Murdoch 1985). Sedaxane (FRAC code 7) is a recently developed fungicide in the succinate dehydrogenase inhibitor family; this fungicide is very effective against *R. solani* (Ajayi and Bradley 2014; Zeun et al. 2013) and is being incorporated into seed treatment formulations for a wide range of crops, including cereal grains, maize, and soybean. Maize seed treatments also are available with activity against *P. penetrans* and other nematodes, and the most commonly used active ingredient is abamectin, a broad-spectrum neurotoxic nematicide in the avermectin family (Bai and Ogbourne 2016; Cochran et al. 2007). Abamectin has been shown to reduce *P. penetrans* populations in maize roots, leading to increased yields (Cabrera et al. 2009; Cochran et al. 2007; MacGuidwin 2010).

The objectives of this study were to assess the impact of seed treatment combinations (including abamectin–fungicide combinations)

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on maize seedlings coinfected with *P. penetrans* and *R. solani*, and to measure interactions between *P. penetrans* and *R. solani* causing seedling disease symptoms on maize.

Materials and Methods

Greenhouse and growth-chamber experiments were conducted with potting media infested with *R. solani* and *P. penetrans* alone or in combination, using maize seed treated with different combinations of seed treatment active ingredients, including commercial formulations of fungicides, a nematicide, and an insecticide. Seed treatment combinations and soil infestation treatments differed between the greenhouse and growth-chamber experiments. All experiments were conducted using *R. solani* isolate 65L-2, AG 2-2 (Liu and Sinclair 1991), provided by Dr. Wayne Pedersen and Dr. Carl Bradley (University of Illinois and University of Kentucky, respectively), and *P. penetrans,* provided by Dr. A. E. MacGuidwin (University of Wisconsin, Madison).

Greenhouse experiment. An experiment was conducted twice in the Department of Plant Pathology and Microbiology greenhouse at the Iowa State University, Ames. Inoculum of *R. solani* was prepared for the greenhouse experiment 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. A culture of *R. solani* was grown on potato dextrose agar at 20°C in the dark. Mycelium from one 7-day-old culture (in a 9-cm Petri dish) was cut into small pieces and mixed in each bag with the substrate mixture. The bags were 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 *R. solani* inoculum. The proportion by volume was 30% inoculum and 70% sand-soil mixture. Cone-tainers were filled with the mixture. A small piece of paper towel was placed in the bottom of each cone-tainer to slow drainage. One maize seed was placed in each cone-tainer. *P. penetrans* was cultured monoxenically (Layne and MacGuidwin 1994) on excised sweet corn 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 water suspension (Martin et al. 1982) in a total volume of 50 ml, which was then diluted to achieve a density of 2,000 nematodes/ml. Nematodes were added to the cone-tainers by injecting 2 ml of suspension (4,000 nematodes) using a microliter pipette at the time of planting (Saeed et al. 1999).

A full factorial experimental design was used in the greenhouse experiment. Experimental factors were seed treatment (8 treatments), nematode infestation (infested or not infested), and fungal infestation (infested or not infested), for a total of 32 treatment combinations. Seed of one maize hybrid (NK Brand hybrid N40T-GT; Syngenta Seeds, Golden Valley, MN) were treated with seven different combinations of active ingredients and a nontreated control was included (Table 1). Seed treatments were used at the recommended commercial rates. Treatments were arranged in a complete randomized block design with six replicate blocks (192 experimental units). An experimental unit was a single plant growing in a single 4-by-21-cm, 150-ml-capacity, plastic, cone-shaped cone-tainer (Stuewe and Sons, Corvallis, OR). Artificial light for 14 h/day supplemented the natural lighting and the temperature mean was 28°C (±8°C). The plants were watered once a day (20 ml/plant) and fertilized once a week, using Peters Excell water-soluble fertilizer (15-5-15).

Growth-chamber experiment. A growth-chamber experiment was conducted twice using rice hulls infested with *R. solani* as fungal inoculum. Inoculum was prepared by incubating sterilized rice hulls with mycelium of *R. solani* for 1 week, then air drying. Inoculum was mixed with autoclaved field soil (3% inoculum by weight) and the mix was used to fill sterilized cone-tainers. Noninfested controls received sterilized rice hulls. *P. penetrans* suspensions were prepared and added to the soil at the time of planting, as described for the greenhouse experiment.

A partial factorial treatment design was used in the growth chamber with nine different treatment combinations. Maize seed treated with abamectin or sedaxane, alone or in combination, were planted in pathogen-free autoclaved field soil or autoclaved field soil infested with *R. solani, P. penetrans*, or both (Table 2). Treatments were arranged as a randomized complete block design with six replicate blocks (54 experimental units). Each cone-tainer was an experimental unit. A constant temperature of 22°C (±0.1°C) was chosen to facilitate maize seedling growth and nematode reproduction. Relative humidity was maintained at 70 to 80%. Light was supplied by cool white fluorescent and incandescent lamps with a photoperiod of 14 h. The plants were watered once a day (20 ml/plant).

Data collection (greenhouse and growth-chamber experiments). Four replicates were harvested 30 days after planting. Plants were removed from the cone-tainers and the roots were washed thoroughly. Shoot lengths (flag leaf) and fresh weights of shoots and roots were measured.

Table 2. Treatment combinations used for growth-chamber experiments on efficacy of maize seed treatment combinations for protection against *Rhizoctonia solani* and root-lesion nematode (*Pratylenchus penetrans*)

Treatment	Infestation		Seed treatment active ingredient (rate)
	P. penetrans	*R. solani*	
1	…	…	Untreated
2	X	…	Untreated
3	X	…	Abamectin (0.20 mg a.i./seed)
4	…	X	Untreated
5	…	X	Sedaxane (0.05 mg a.i./seed)
6	X	X	Untreated
7	X	X	Abamectin (0.20 mg a.i./seed)
8	X	X	Sedaxane (0.05 mg a.i./seed)
9	X	X	Abamectin (0.20 mg a.i./seed) + sedaxane (0.05 mg a.i./seed)

Table 1. Seed treatments used in a greenhouse experiment with maize seedlings grown in sand-soil mixture infested with *Rhizoctonia solani* or *Pratylenchus penetrans*[y]

Treatment	Active ingredients[z]	Chemical group	Formulation (%)	Brand name	Rate
1 (FMA)	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 + TB	Benzimidazole	42.3	MaximQuattro	20 g/100 kg
3	FMA + TM	Neonicotinoid	47.6	Cruiser	0.25 mg/seed
4	FMA + TM + TB	…	47.6	CruiserMaxx	
5	FMA + AB	Avermectin	46.3	Avicta	0.25 mg/seed
6	FMA + AB +TB	…	12.4	…	…
7	FMA + AB + TM +TB	…	12.4	…	…
8	Untreated	…	0	…	…

[y] Treatment 1 is the baseline formulation and treatments 2 through 7 include fludioxonil, mefenoxam, and azoxystrobin (FMA) at the same rates as treatment 1.
[z] Ingredient abbreviations: TB = thiabendazole, TM = thiamethoxam, and AB = abamectin.

To analyze root morphology for each treatment, roots were scanned and image analyses were conducted with WinRhizo 2008a software (Regent Instruments Inc.). The procedures were as follows: washed and intact roots were spread out in a transparent tray in order to minimize overlapping of roots during scanning process. A blue plastic sheet served as the image background. Image recording was performed at a resolution of 600 dpi using a 24-bit color mode, and images were saved as TIFF (tagged image file format) files. All other scanner settings, such as dust removal and so on, were turned off. A Dell Precision T3500 computer was used to operate the scanner (an Epson Perfection V700 Photo–Dual Lens System). The following root morphology characteristics were determined: total root length (in centimeters), total surface area (in square centimeters), total volume (in cubic centimeters), number of root tips, number of root forks, and length (in centimeters) of fine roots (<0.5 mm in diameter). After scanning, shoots and roots were oven dried at 110°C for 2 days and weighed. Treatments in the growth-chamber experiments were ranked from 1 to 9 according to each variable, with 1 assigned to the treatment with the highest mean and 9 assigned to the treatment with the lowest mean. Ranks were assigned for each replicate block, and the mean rank among the measured variables for each treatment was then calculated for both experiments combined.

Two replicates per treatment were harvested after 6 weeks in order to extract nematodes from soil and roots. A 100-cm^3 soil sample was collected after the soil was removed from the pots, thoroughly mixed, and assayed for *P. penetrans* with a centrifugal flotation technique (Jenkins 1964). Additionally, roots were cut into 1-cm-long pieces, mixed, and incubated in Baermann funnels for 2 days (Viglierchio and Schmitt 1983). After nematodes were collected, the roots were dried at 100°C for 2 days and weighed. The number of juvenile and adult *P. penetrans* was counted using an inverted compound microscope. The total number of *P. penetrans* present per cone-tainer was calculated based on soil volume and root weight. The number of *P. penetrans* per gram of dry root weight was also determined.

Statistical analysis. The data were combined for the two runs of the greenhouse experiment because treatment effects were similar. Greenhouse data were analyzed by analysis of variance (ANOVA) for a randomized complete block design (SAS Inc., Cary, NC), testing the main effects and interactions of seed treatment, *R. solani* infestation, and *P. penetrans* infestation. In order to meet ANOVA assumptions regarding normality and homogeneity of variance, ANOVA were performed on transformed ($\log_{10}$) data, except for nematode counts. Analysis of all main effects and interactions was conducted using all treatment combinations. Seed treatment effects also were analyzed for infested treatments only (excluding the noninfested treatments, which did not display any seed treatment effects). Seed treatment means were analyzed separately by pathogen treatment: fungus-infested plants alone and *P. penetrans*-infested plants alone. Least significant differences (LSD; $P \leq 0.05$) for comparing treatment means were calculated according to the GLM procedure of SAS.

Growth-chamber experiment data were analyzed separately for the two runs of the experiment because of variable effects of *P. penetrans* infestation between the runs. Because of the unbalanced design of the growth-chamber experiment, a factorial analysis was not conducted; however, ANOVA was conducted (PROC GLM) for main treatment effects and mean comparisons were made based on treatment comparisons according to LSD values. Treatments also were ranked (1 to 9, from highest to lowest means), and mean ranks for each of the nine treatments were compared according to Welch's weighted-variance one-way ANOVA.

Results

Greenhouse experiments. Infestation with *R. solani* had greater effects than nematode infestation, causing statistically significant reductions in root length, root volume, number of root tips, number of root forks, surface area, and number of fine roots (Table 3). Nematode infestation significantly reduced only shoot fresh weights (Table 3). Seed treatment significantly affected most of the measured variables. There was a significant interaction between seed treatment and nematode infestation affecting root dry weight, numbers of root tips, and the number of root forks (Table 3). The effects on the remaining variables were not statistically significant.

ANOVA on *R. solani*-infested plants indicated significant seed treatment effects for root length, root volume, number of root tips, number of root forks, root surface area, and number of fine roots but not for the other variables (Table 4). Data are not shown for root volume or number of root tips, which were highly correlated with other variables. Regarding the nematode-infested plants, ANOVA indicated significant seed treatment effects for shoot fresh weight, shoot dry weight, root fresh and dry weights, root length, root volume, number of root tips, number of root forks, root surface area, and length of fine roots (Table 4). Data are not shown for dry weights, root volume, or number of root tips, which were highly correlated with other variables. Treatments 6 (fludioxonil, mefenoxam, and azoxystrobin [FMA] + abamectin + thiabendazole) and 7 (FMA + abamectin + thiamethoxam + thiabendazole) resulted in the highest means for most of the plant health variables, followed by treatments 2 (FMA + thiabendazole) and 4 (FMA + thiamethoxam + thiabendazole) (Table 4).

Nematode counts from soil and roots were low, ranging from 3.6 to 47.2 nematodes 100 cm^{-3} of soil and from 1.5 to 6.7 nematodes g^{-1} of root tissue. Fungal infestation significantly increased the number of nematodes in soil ($P = 0.002$) and decreased the number in roots ($P = 0.03$), and there also was a significant interaction between seed treatment and fungal infestation ($P = 0.0006$) affecting numbers of nematodes in the soil. There were more nematodes recovered from soil for treatment 6 than the untreated control but other seed treatments did not affect nematode numbers in soil or roots (Table 5).

Growth-chamber experiments. There were significant differences among treatments for shoot length and fresh weight, root fresh weight, root length, volume, surface area, numbers of root forks, and the length of fine roots; however, effects on numbers of root tips and nematodes recovered from roots and soil were not significant (Tables 6 and 7). *R. solani* infestation caused significant reductions in most of the measured variables compared with the noninfested control but *P. penetrans* infestation did not significantly reduce these variables, and the combined infestation did not differ significantly from the *R. solani* infestation in most cases. In treatments infested with *R. solani* alone, nearly all measures of root and shoot development were significantly greater in plants grown from seed treated with sedaxane. In treatments infested with *P. penetrans* alone, seed treatment with abamectin significantly improved shoot and root fresh weights, root volume and surface area, and the number of root forks in one of the two runs of the experiment (Tables 6 and 7).

In treatments infested with a combination of both pathogens, sedaxane seed treatment significantly increased shoot length and fresh weight in both runs of the experiment and also increased root volume, length of fine roots, and number of root forks in one of two runs of the experiment (Tables 6 and 7). In the combined infestations, seed treatment with abamectin alone did not significantly affect seedling growth and development; however, seed treatment with sedaxane and abamectin together significantly increased shoot length and fresh weight, root surface area, number of root forks, and length of fine roots in both runs of the experiment, and increased root length and fresh weight in one of two runs of the experiment (Tables 6 and 7).

The numbers of nematodes recovered from soil and roots were less than 250 per cone-tainer, and there were no significant treatment effects for either soil or root population densities in either run of the experiment (Tables 6 and 7).

Ranking of treatments (1 to 9, from highest to lowest means) in the growth-chamber experiment revealed that untreated seed planted into *R. solani*-infested soil had the lowest ranking (poorest shoot and root development), with a mean ranking of 8.77 of 9, which was significantly worse than any other treatment. Sedaxane-treated seed planted in *R. solani*-infested soil had the highest ranking (best shoot and root development), with a mean of 1.64, significantly better than any other treatment. The second-best mean ranking (3.18) occurred with seed treated with both abamectin and sedaxane and grown in soil infested with both *R. solani* and *P. penetrans*; this treatment was

significantly better than all other treatments except the sedaxane–*R. solani* treatment (Fig. 1).

Discussion

In both greenhouse and growth-chamber experiments, infestation of the potting medium with *R. solani* or *P. penetrans* resulted in significant detrimental effects on seedling and root health variables measured in this study. However, the effects of *R. solani* were far more extensive and severe than those of *P. penetrans* in both types of experiment. In the greenhouse experiment, *R. solani* had significant negative effects on all root variables. In the growth-chamber experiment, the treatment with *R. solani* infestation and untreated seed was consistently ranked

Table 3. *P* values for effects of seed treatments (ST) and pathogen infestations on seedling health variables for maize plants growing in sand-soil mixture infested with *Rhizoctonia solani* (R) or *Pratylenchus penetrans* (P) or both in a greenhouse experiment[z]

Variables	ST	R	P	ST × R	ST × P	R × P	ST × R × P
Shoot length (cm)	ns	ns	ns	ns	ns	ns	ns
Shoot fresh (g)	0.01	ns	0.02	ns	ns	ns	ns
Shoot dry (g)	ns	ns	ns	ns	ns	ns	ns
Root fresh (g)	0.04	ns	ns	ns	ns	ns	ns
Root dry (g)	ns	ns	ns	ns	0.05	ns	ns
Root length (cm)	0.006	<0.0001	ns	ns	ns	ns	ns
Root volume (cm^3)	ns	<0.0001	ns	ns	ns	ns	ns
Root tips	0.01	<0.0001	ns	ns	0.04	ns	ns
Root forks	0.004	<0.0001	ns	ns	0.04	ns	ns
Root surface area (cm^2)	0.008	<0.0001	ns	ns	ns	ns	ns
Fine roots (cm)	0.01	<0.0001	ns	ns	ns	ns	ns

[z] Analysis of variance was conducted with $\log_{10}$-transformed data. Data are combined for two runs, with four replications each for each treatment combination. ns = not significant ($P > 0.05$).

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 *Rhizoctonia solani* or *Pratylenchus penetrans* in a greenhouse experiment[x]

Infestation, treatment[y]	Seed treatment active ingredients[z]	Shoot FW (g)	Root length (cm)	Root FW (g)	Area (cm^2)	Root forks (*n*)	Fine roots (cm)
R. solani							
1	FMA	0.90 a	91.2 ab	0.90 a	21.2 bc	450 bc	42.9 b
2	FMA + TB	1.03 a	98.8 a	0.92 a	23.1 abc	483 bc	44.3 b
3	FMA + TM	0.92 a	95.5 ab	0.94 a	23.3 abc	578 abc	44.9 b
4	FMA + TM + TB	0.99 a	96.3 a	0.96 a	24.0 ab	537 abc	44.3 b
5	FMA + AB	1.04 a	107.3 a	1.17 a	26.0 ab	649 ab	45.4 b
6	FMA + AB + TB	1.12 a	104.8 a	0.99 a	28.0 ab	605 ab	47.6 b
7	FMA + AB + TM + TB	1.00 a	121.8 a	1.04 a	30.7 a	764 a	80.9 a
8	Untreated	0.82 a	59.8 b	0.79 a	14.4 c	314 c	27.7 b
P. penetrans							
1	FMA	0.76 bc	136.4 ab	0.77 b	30.8 ab	824 ab	61.8 bc
2	FMA + TB	0.98 abc	149.2 ab	1.02 ab	35.8 ab	897 ab	72.0 abc
3	FMA + TM	0.76 bc	136.7 ab	0.89 ab	35.2 ab	844 ab	78.9 abc
4	FMA + TM + TB	0.83 abc	164.4 a	1.02 ab	38.9 ab	999 ab	72.7 abc
5	FMA + AB	1.03 abc	167.4 a	1.02 ab	40.9 a	1,334 a	87.3 ab
6	FMA + AB + TB	1.16 a	175.8 a	1.10 ab	46.2 a	1,140 a	88.4 ab
7	FMA + AB + TM + TB	1.10 ab	193.9 a	1.33 a	44.2 a	1,183 a	101.8 a
8	Untreated	0.70 c	94.7 b	0.69 b	23.5 b	582 b	43.6 b

[x] Values are means of 16 observations from two runs of the experiment. FW = fresh weight and Area = root surface area. 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] There was no *R. solani–P. penetrans* interaction for any of the response variables measured; therefore, *R. solani*-infested treatments include all those that were infested with *R. solani* (four replications with the fungus alone and four replications infested with both pathogens). Likewise, *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] Ingredient abbreviations: FMA = fludioxonil + mefenoxam + azoxystrobin, TB = thiabendazole, TM = thiamethoxam, and AB = abamectin.

Table 5. *Pratylenchus penetrans* adult and juveniles recovered from soil and roots of maize seedlings grown from seed treated with different seed treatment products and grown in a sand-soil mixture infested with *P. penetrans* in a greenhouse experiment[y]

Treatment	Seed treatment active ingredients	Nematodes 100 cm^{-3} of soil	Nematodes g^{-1} of root[z]
1	Fludioxonil + mefenoxam + azoxystrobin (FMA)	3.6b	3.3
2	FMA + thiabendazole	9.7b	1.5
3	FMA + thiamethoxam	21b	1.5
4	FMA + thiamethoxam + thiabendazole	11.8b	5.2
5	FMA + abamectin	9.8b	3.7
6	FMA + abamectin + thiabendazole	47.2a	4.8
7	FMA + abamectin + thiamethoxam + thiabendazole	14.1b	6.7
8	Untreated	19.5b	3.3

[y] Values are means of eight observations from two runs of the experiment. No nematodes were recovered from noninfested treatments. Values within a column and infestation treatment followed by the same letter are not significantly different according to Fisher's least significant difference ($\alpha = 0.05$).

[z] No significant difference.

Table 6. Mean shoot length (centimeters) and fresh weight (FW, grams); root FW (grams), length (centimeters), volume (cubic centimeters), tips (number), forks (number), surface area (square centimeters), and fine roots (centimeters); and nematodes in soil (nematodes-100^{-3} of soil) or roots (nematodes-g^{-1} of root tissue) for maize seedlings grown from seed treated with sedaxane, abamectin, or both and exposed to *Rhizoctonia solani* (Rs), *Pratylenchus penetrans* (Pp), or both in run 1 of the growth-chamber experiment[y]

			Shoot		Root							Nematodes	
Rs	Pp	Seed treatment	Length	FW	FW	Length	Volume	Tips	Forks	Area	Fine	Soil	Root
–	–	None	24.3	1.34	0.53	72.6	0.40	64.5	249.8	18.8	28.4	…	…
–	+	None	21.7	1.09	1.32	143.5	0.94	123.3	435.3	41.0	40.6	24.5	38.5
–	+	Abamectin	26.9	1.53	0.71	65.7	0.53	63.0	267.3	20.9	27.0	34.5	7.5
+	–	None	6.9	0.40	0.23	23.1	0.19	26.3	66.3	7.3	6.6	…	…
+	–	Sedaxane	40.0	4.06	1.65	168.1	1.08	144.3	715.0	47.5	60.4	…	…
+	+	None	13.7	0.77	0.59	45.9	0.37	39.0	87.5	14.6	9.4	72.0	93.0
+	+	Abamectin	12.4	0.77	0.27	30.3	0.16	30.5	99.0	7.8	10.5	94.5	14.0
+	+	Sedaxane	31.5	2.04	0.71	76.4	0.47	64.3	264.8	21.3	25.0	112.5	60.0
+	+	Sedaxane + abamectin	34.9	2.39	0.92	98.8	0.59	101.0	382.0	26.9	37.1	96.5	0.0
…	…	LSD[z]	10.0	0.82	0.39	45.5	0.24	NS	169.6	12.3	14.0	NS	NS

[y] Data are only for emerged plants; plants failing to emerge were considered missing data. Abamectin was applied to seed at a rate of 0.20 mg a.i./seed and sedaxane was applied at 0.05 mg a.i./seed. NS = not significant.
[z] Least significant difference ($\alpha = 0.05$).

Table 7. Mean shoot length (centimeters) and fresh weight (FW, grams); root FW (grams), length (centimeters), volume (cubic centimeters), tips (number), forks (number), surface area (square centimeters), and fine roots (centimeters); and nematodes in soil (nematodes-100^{-3} of soil) or roots (nematodes-g^{-1} of root tissue) for maize seedlings grown from seed treated with sedaxane or abamectin or both and exposed to *Rhizoctonia solani* (Rs), *Pratylenchus penetrans* (Pp), or both in run 2 of the growth-chamber experiment[y]

			Shoot		Root							Nematodes	
Rs	Pp	Seed treatment	Length	FW	FW	Length	Volume	Tips	Forks	Area	Fine	Soil	Root
–	+	None	21.1	0.93	0.44	38.9	0.33	27.8	57.5	12.6	7.8	65.5	27.0
–	+	Abamectin	26.5	1.61	0.81	69.7	0.60	65.3	122.8	22.8	12.3	205.0	7.5
+	–	None	5.0	0.09	0.32	29.5	0.23	30.8	78.0	9.1	9.3	…	…
+	–	Sedaxane	31.8	2.35	0.91	103.0	0.69	68.8	235.0	29.7	29.8	…	…
+	+	None	13.0	0.72	0.57	42.3	0.40	52.3	81.5	14.5	8.3	85.5	26.0
+	+	Abamectin	14.7	0.81	0.59	67.1	0.44	55.5	138.5	18.9	21.1	227.0	11.0
+	+	Sedaxane	22.5	1.40	0.79	72.0	0.63	61.3	108.5	23.7	11.8	93.0	20.0
+	+	Sedaxane + abamectin	27.0	1.75	0.80	81.3	0.59	78.5	187.5	24.5	21.4	168.5	5.5
…	…	LSD[z]	7.9	0.51	0.23	40.2	0.21	NS	65.8	9.7	10.1	NS	NS

[y] Data are only for emerged plants; plants failing to emerge were considered missing data. Abamectin was applied to seed at a rate of 0.20 mg a.i./seed and sedaxane was applied at 0.05 mg a.i./seed. NS = not significant.
[z] Least significant difference ($\alpha = 0.05$).

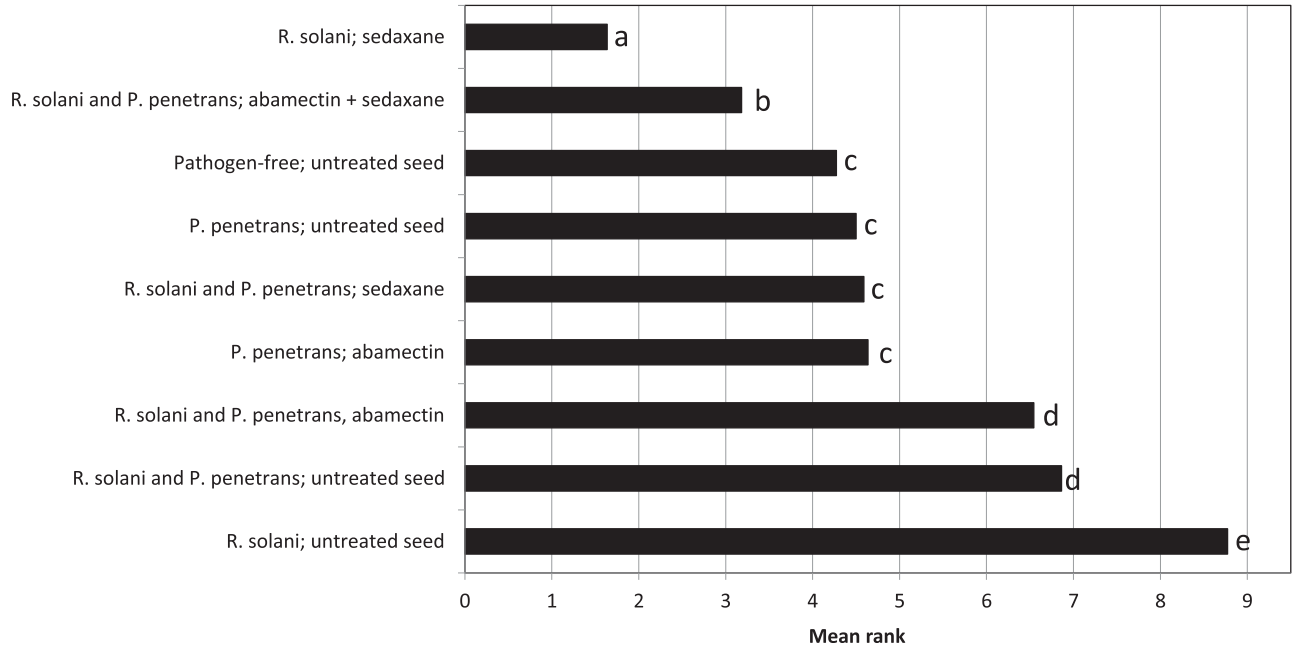

Fig. 1. Mean rank (1 to 9, from best to worst) of treatment combinations for variables related to shoot health and root health for maize seedlings grown from seed treated with abamectin or sedaxane (or both) and planted in soil infested with *Rhizoctonia solani* or *Pratylenchus penetrans* (or both) in a growth chamber experiment. Mean separation was conducted according to Fisher's least significant difference test results from Welch's weighted-variance one-way ANOVA.

worst for all the measured variables. Root image analysis with WinRhizo software provided quantitative documentation of the aggressive nature of the *R. solani* isolate used in the study. These results are similar to other recent studies with *Rhizoctonia* spp. on other crops. Paulitz et al. (2003) and Schroeder and Paulitz (2008) documented severe root damage caused by *Rhizoctonia* spp. in wheat and barley, and reported the advantages of WinRhizo root scanning technology compared with other measures of root damage.

The limited effects of *P. penetrans* on seedling variables might be attributed to poor root colonization by the nematodes. The number of *P. penetrans* nematodes recovered from soil and from the maize roots was lower than expected (less than 250 nematodes/plant), and there were few differences among nematode-infested treatments. In previous studies on *P. penetrans*, low recovery of nematodes from roots also has been observed (da Silva et al. 2016; Rotenberg et al. 2004). *R. solani* inoculum made with corn meal for the greenhouse experiments may have a detrimental effect on the ability of the nematodes to infect and reproduce (G. L. Tylka, personal communication). Variable greenhouse temperatures, as well as infestation methods and the ratio of the two pathogens, may have affected nematode activity and results of the nematode-infested treatments. The naturally occurring ratio of pathogen populations is difficult to estimate, and additional experimentation with varying ratios of *R. solani*/*P. penetrans* inoculum might reveal more significant effects of the nematode. The relatively minor effects of *P. penetrans* infestation compared with the effects of *R. solani* infestation probably limited our ability to detect seedling health improvements due to abamectin seed treatment. In treatments that were infested with *P. penetrans*, treatment 7 (which included all six active ingredients, including abamectin) was consistently the best treatment. The seed treatment combinations that included abamectin (5, 6, and 7 in the greenhouse experiment) were always among the best treatments; however, they typically did not differ significantly from the corresponding treatments lacking abamectin (greenhouse treatments 1, 2, and 4). Treatments that included abamectin had fewer nematodes extracted from roots than the untreated controls; however, these differences were not significant. In the growth-chamber experiment, abamectin significantly improved shoot weight and several of the root variables in *P. penetrans*-infested treatments in the second run of the experiment but not the first. In treatments infested with both pathogens, the addition of abamectin alone did not result in significant improvements in shoot and root variables, probably because most of the damage was caused by *R. solani* in those treatments. However, the combined sedaxane-abamectin seed treatment ranked significantly better than the sedaxane-alone treatment for plants infested with both pathogens (Fig. 1), demonstrating the added value of abamectin under these conditions.

Several reports have been published related to the interaction between a plant-parasitic nematode and *R. solani*. LaMondia and Martin (1989) reported that black root rot of strawberry, caused by *Rhizoctonia* spp., was more severe in the presence of *P. penetrans*. However, to our knowledge, our study is the first to investigate interactions between *P. penetrans* and *R. solani* on maize. Evidence of significant fungus–nematode interactions in this study was lacking. In the greenhouse experiment, there were no statistically significant interactions between these two experimental factors. The design of the growth-chamber experiments did not allow for a statistical test of interaction but the combined infestation treatment did not show seedling damage significantly different from the *R. solani* infestation treatment. Temperature can be critical in some nematode–fungus interactions (France and Abawi 1994; Walker et al. 2000). In order to fully understand root-lesion nematode interactions with seedling pathogens, these studies should be repeated under a range of temperatures. In our study, it is likely that the severe damage caused by *R. solani* left little root tissue available for colonization by *P. penetrans*, limiting the opportunity for additional damage. This possibility is supported by the fact that, when *R. solani* damage was controlled through the use of sedaxane in the growth-chamber experiment, the addition of *P. penetrans* resulted in significant reductions in shoot and root growth and development (Fig. 1; Tables 5 and 6).

Seed treatments resulted in significant improvements in shoot and root variables compared with the infested, untreated controls in both the greenhouse and the growth-chamber experiments in nearly all cases. Differences among the seed treatment combinations were less evident. The greenhouse experimental design allowed for comparisons to assess the impacts of individual components (abamectin, thiabendazole, and thiamethoxam) of the seed treatment combinations. Thiabendazole is a broad-spectrum fungicide that is primarily used for protection against *Fusarium* spp. It also has been reported to be effective as a potato seed treatment for protection against *Rhizoctonia* spp. (Leach and Murdoch 1985). In our study, the addition of thiabendazole to the baseline formulation (FMA) resulted in numerically higher shoot and root development measures but none of these differences was significant. Fludioxonil and azoxystrobin have activity against *R. solani* and seem to have provided adequate protection against this fungus, so that the addition of thiabendazole did not produce results significantly different from the baseline formulation.

In addition to providing insect control, thiamethoxam is reported to affect seedling growth and promote defense responses in maize and other plant species (Afifi et al. 2015; Macedo et al. 2013). As with thiabendazole, in our study, the addition of thiamethoxam to the baseline formulation (FMA) resulted in numerically higher shoot and root development measures but none of these differences was significant. When both thiabendazole and thiamethoxam were added to the baseline formulation, shoot and root variables showed numerical but not statistically significant increases. Plant-growth-enhancing effects of thiamethoxam likely are influenced by the environment, and conditions in our experiments may not have been conducive to their expression.

Sedaxane was included as a treatment only in the growth-chamber experiment. Seed treatment with this fungicide caused dramatic improvements in the health of seedlings grown in the *R. solani*-infested potting media. All shoot and root growth and development variables were significantly improved with the use of sedaxane whereas the untreated, *R. solani*-infested treatment had the lowest means for all variables. In most cases, the sedaxane-treated, *R. solani*-infested treatment had the highest means. For many of the variables, the sedaxane-treated, *R. solani*-infested treatment had significantly higher means than the untreated, noninfested control, which suggests that sedaxane had a positive effect on plant growth and development in addition to its activity against *R. solani*. This effect has been reported previously for sedaxane seed treatment of wheat (Barchietto et al. 2012).

Results of our study reinforce the value of maize seed treatment with combinations of active ingredients that target multiple pathogens. Although we did not observe strong evidence for an interaction between the fungus and nematode, it was clear that seed treatment combinations with active ingredients targeting both *R. solani* and *P. penetrans* provided the best results when both organisms were present. Sedaxane demonstrated very effective control of *R. solani* and results also indicated that it may have enhanced seedling growth and development in addition to its fungicidal activity. Additional experiments are needed to confirm whether this effect occurs in the absence of pathogen attack.

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