

**Interactions between lesion nematodes and corn pathogens**

by

**Marcos Paulo da Silva**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
**MASTER OF SCIENCE**

Major: Plant Pathology

Program of Study Committee:

Gary Munkvold, Major Professor

Alison Robertson

Roger Elmore

Iowa State University

Ames, Iowa

2010

Copyright © Marcos Paulo da Silva, 2010. All rights reserved.

## TABLE OF CONTENTS

ABSTRACT	iii
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW	1
Thesis organization	1
Literature review	1
Research objectives	12
Literature cited	12
CHAPTER 2. LESION NEMATODE INTERACTIONS BETWEEN <i>FUSARIUM GRAMINEARUM</i> AND <i>FUSARIUM VERTICILLIOIDES</i> ON MAIZE SEEDLINGS	22
Abstract	22
Introduction	24
Material and methods	27
Results	30
Discussion	32
Literature cited	37
Tables	42
Figures	45
CHAPTER 3. LESION NEMATODE INTERACTIONS BETWEEN <i>PYTHIUM ULTIMUM</i> AND <i>RHIZOCTONIA SOLANI</i> ON MAIZE SEEDLINGS	64
Abstract	64
Introduction	65
Material and methods	69
Results	72
Discussion	74
Literature cited	78
Tables	84
Figures	87
CHAPTER 4. GENERAL CONCLUSION	106
ACKNOWLEDGMENTS	108

## ABSTRACT

Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites of a variety of hosts worldwide. They are among the most common parasitic nematodes that feed on maize roots at all plant growth stages. Maize seedlings also are commonly attacked by pathogenic fungi and Oomycetes. The combination of nematode and fungus often results in a synergistic interaction wherein the crop loss is greater than expected from either pathogen alone or an additive effect of the two together. These interactions have been described from several crops, but lesion nematode-seedling pathogen interactions on maize (*Zea mays* L.) have not been intensively studied. Developments in seed treatment technology now offer new tools for the study and management of nematode-fungus interactions. The objectives of this study were to measure interactions between *Pratylenchus penetrans* and fungal/oomycete pathogens (*Fusarium graminearum*, *Fusarium verticillioides*, *Pythium ultimum* and *Rhizoctonia solani*) causing seedling diseases in maize; assess the impact of nematode control with abamectin on these interactions and evaluate added benefit of abamectin combined with fungicide seed treatment for seedling disease management. *Pythium ultimum*, *Fusarium graminearum* and *Fusarium verticillioides* experiments were conducted twice each in a growth chamber and *Rhizoctonia solani* experiments were conducted twice in the greenhouse at the Iowa State University, Ames, Iowa. Experiments were conducted in 150 ml pots that were filled with an autoclaved sand-soil mixture combined with inoculum of *Fusarium graminearum*, *Fusarium verticillioides*, *Pythium ultimum* or *Rhizoctonia solani* (colonized corn meal/sand mixture). A suspension of 4000 *P. penetrans* (adults and

juveniles) was added to the pots at the time of planting. A factorial experimental design was used including 8 seed treatments x 4 pathogen combinations x 6 replicates. Four replicates of each treatment were harvested 30 days after planting. Shoot lengths, fresh and dry shoot and root weights, were determined. Digital images of the root systems were recorded with a flatbed scanner and image analysis conducted with WinRhizo software (Regent Instruments Inc.); root length, volume, tips, branching, surface area, discoloration and diameter class distribution were determined. Two replicates were harvested 42 days after planting and nematodes from soil and roots were extracted and counted. The results demonstrated significant effects on root health with interactions between fungal or Oomycete pathogens and nematodes. Seed treatments showed efficacy against fungal and nematode inoculation, improving most measures of seedling health compared to the nontreated control; mainly those seed treatment combinations including abamectin or abamectin with thiabendazole. Root structure analysis from WinRhizo showed that seed treatment significantly improved root system characteristics such as root volume, root length, number of tips, forks, surface area, fine roots and reduced diseased root length and diseased root volume. Fungal inoculation had a stronger effect compared to nematode inoculation, although diseased root length and diseased root volume were significantly affected by nematode inoculation, and seed treatment combinations with abamectin significantly reduced diseased root length and volume when compared to the non-treated check. Abamectin in combination with commercial seed treatment fungicides significantly reduced lesion nematode infection of the maize root system. This study provides the first quantitative evidence of interactions between *P. penetrans* and maize seedling pathogens in relation to root and seedling health. Overall,

seed treatments with abamectin in combination with fungicides, provided the best control of seedling disease symptoms and also nematode feeding.

## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### **Thesis organization**

This thesis is organized into 3 chapters. Chapter one contains the introduction, literature review, and research objectives. The second chapter will discuss lesion nematode interactions between *Fusarium verticillioides* and *Fusarium graminearum* on maize seedlings. The third chapter will describes lesion nematodes interaction with *Pythium ultimum* and *Rhizoctonia solani* on maize seedlings.

#### **Literature review**

Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites of a variety of hosts worldwide. They are known as root-lesion nematodes due to the necrotic lesions that they cause on host roots. They can be found in all agricultural regions of the world and cause severe yield damage to the crops due the feeding on the root tissues (W.K. 1993; Castillo and Vovlas 2007). Furthermore, root-lesion nematodes are probably the most important nematodes attacking corn (Norton 1983; Norton 1984; Windham 1998).

There are several species of root-lesion nematodes that can be associated with corn in Iowa, but the most important ones are *P. hexincisus*, *P. penetrans* and *P. scribneri* (Norton 1983; Norton 1984; Windham 1998). Root-lesion nematodes also have been reported to cause significant damage to sweet corn in field microplots and in fields (Olthof and Potter 1973).

*Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven (Burpee and Bloom 1978; Martin, Riedel et al. 1982; Rowe, Riedel et al. 1985; Huan, Santo et al. 1988), is one of the most common *Pratylenchus* species, with a wide range of hosts around the world and a cosmopolitan distribution throughout temperature regions (Corbett 1973; Mai, Bloom et al. 1977; Loof 1991). *Pratylenchus penetrans* can reduce both crop yield and quality (Olthof and Potter 1973; Seinhorst 1998). It has been recorded on over 350 hosts mainly in temperate areas in Europe, North America, Central and South America, Africa, Asia and Australia (Corbett 1974). It is a major pathogen of fruit and conifer nurseries in many areas and causes serious losses in tobacco, apple and cherry orchards and in roses (Corbett 1973). Growth reduction is frequently observed in several crops (Olthof and Potter 1973; Seinhorst 1998). *P. penetrans* has been reported as a pathogen of many crops, such as legumes (Townshend 1978; Elliott and Bird 1985), vegetables (Townshend 1963a; Hung and Rohde 1973), strawberries (Townshend 1963b), fruits (Mountain and Patrick 1959; Pitcher, Patrick et al. 1960), corn and potato (Dickerson, Darling et al. 1964) and turf grasses (Troll and Rohde 1966).

*P. penetrans* is probably one of the most common species found on corn in the United States (Norton 1984) and on sweet corn in Eastern Canada (Potter and Townshend 1973). *P. penetrans* damage can be easily overlooked or mistaken because the above ground symptoms are non-specific and difficult to distinguish from other factors, such as poor fertility of the soil, lack of moisture, weather, soil pathogens or some other causes. Furthermore, high densities of nematodes usually exist in patches, so the damage is most noticeable as round to oval patches of stunted and chlorotic (yellowish) and thin plants in the fields (Norton 1983; Norton 1984; Windham 1998).

### **Feeding behavior and mechanism of pathogenesis**

*Pratylenchus penetrans* is a destructive migratory endoparasite that enters and migrates within roots and feed on various tissues, resulting in necrotic lesions on the root surface. As a result, infections can appear along the entire root length of the host plant, excluding root tips (Townshend and Stobbs 1981; Castillo, Vovlas et al. 1998); root lesions can fuse and become discolored with time, although the color of the lesion varies with the host (Townshend and Stobbs 1981).

According to Zunke (1990), *Pratylenchus penetrans* tend to enter through the roots in the regions of root hair development and also the elongation zone (Troll and Rohde 1966). After that, they migrate between cell walls using their stylets (Zunke 1990). In addition to mechanical force generated through its stylet and body muscles (Zunke 1990), *P. penetrans* also secretes cellulolytic enzymes in order to degrade cell walls (Krusberg 1960; Uehara, Kushida et al. 2001).

Therefore, *P. penetrans* mechanism of pathogenesis is related to the way that the nematode can affect the host by either feeding directly on roots or by interacting with other organisms in disease complexes such as those involving fungi (Endo 1975). In fact, *P. penetrans* process of pathogenicity it is due do the predisposition of plants by nematode wounding roots. As a result of that, there is an increase susceptibility to a successive disease caused by other organisms.

### **Biology and ecology of *Pratylenchus penetrans***

There is a lack of information concerning the true length of *Pratylenchus* life-cycles under field conditions. However, some work has been done in the laboratory in order to estimate the life-cycle in several nematode-host plant combinations. For instance, Turner and

Chapman (1972), working in red clover, discovered that *P. penetrans* completed a generation in 54-65 days and produced 16-35 eggs per female at a rate of 1-2 eggs per day. In contrast, the time required to complete the life-cycle can be considerably different, because it is related and dependent on temperature and moisture. In the particular case of *Pratylenchus penetrans* (Acosta and Malek 1979) reported that optimum temperature is 25°C.

*Pratylenchus penetrans* reproduces sexually (Thistlethwayte 1970). After fertilization, the female lays its eggs singly or in small groups inside the host or in the soil. After embryonic development within the egg to the first-stage juvenile (J1), the nematode molts to the second-stage juvenile (J2) which hatches from the egg. All juvenile and adult life stages of *Pratylenchus* are vermiform and mobile and all life stages (except for the egg and J1) can infect host plants (Castillo, Vovlas et al. 1998).

Unlike other genera such as *Heterodera glycines*, that have a sedentary life style, *P. penetrans* do not become sedentary in the roots and feeding is restricted almost entirely to the root cortex (Loof 1991).

Many factors have been reported to influence the distribution, growth, reproduction, population and development of *P. penetrans*, including soil texture, soil temperature, soil moisture and soil pH (Castillo and Vovlas 2007). Wallace 1973 reported that some species of *Pratylenchus* are more prevalent on lighter sandy soil than with heavier soils. For instance, Florini, Loria et al. (1987), reported that *P. penetrans* was more often found in sandy soils. Another factor is related with soil moisture. Very low or very high soil moisture conditions can suppress *P. penetrans* invasion and reproduction into alfalfa roots (Kable and Mai 1968). However, different types of soil can influence on population density of *P. penetrans*. For

example (Kable and Mai 1968), working with clay loam soil did not find any increasing of nematode population with increasing soil moisture.

Equally important, soil pH plays an important role in nematode development and the optimum pH extent for *Pratylenchus* spp. it is related with the species of host plant (Castillo and Vovlas 2007). Some research has been done in vetch and alfalfa (Morgan and MacLean 1968; Willis 1972) and pointed out that *P. penetrans* does best in the range of pH 5.2-6.4, and reproduction rate can drop when pH reaches 7.

### **Control**

Once introduced into soil, *Pratylenchus penetrans* can be difficult to control and usually cannot be eradicated completely. For this reason, before choosing any management tactic in order to reduce nematode population and damage, precise diagnosis of the species and population levels of *Pratylenchus* should be assessed from soil and also from root samples. Nematode damage thresholds, vary among *Pratylenchus* species, crop value, geographic location and the potential for disease complexes (Castillo and Vovlas 2007). For instance, based on (Dickerson, Darling et al. 1964), working with corn, found that the damage threshold is 0.25 *P. penetrans*/cm<sup>3</sup> soil. Furthermore, according to (Osteen, Moffitt et al. 1988), estimated corn yield losses ranged from 1243 kg ha<sup>-1</sup> to 2284 kg ha<sup>-1</sup> at when soil populations were 100-200 nematodes per 150 cm<sup>3</sup>.

Among available tactics for *Pratylenchus* management, two are typically used: sanitation and chemical nematicides. The best method is to prevent nematode introduction into a field, through plant material or machines.

As mentioned above, once *Pratylenchus penetrans* infests a field it can be difficult and sometimes impossible to eradicate them. Even though some practices can be used in

order to minimize inoculum levels, such as turning over the soil layer to expose infected roots, and using certified free *Pratylenchus* propagation plant material. In addition, crop rotation, host plant resistance, cover crops and other cultural practices should be used in order to minimize damage caused by *P. penetrans* (Duncan and Noling 1998).

In corn, nematicides are still being used despite environment concerns. Nematicides can significantly reduce nematode populations. Applications of 1,3-D and carbofuran combined in Iowa resulted in good control of *P. hexincisus* (Norton and Hinz 1976). Moreover, based on (Rich, Johnson et al. 1985) data from Hamilton County, Florida, Rich, Johnson et al. 1985 reported that using pre-plant treatments with 1,2-D-1,3-D or carbofuran considerably reduced populations of *P. brachyurus* and also *P. zae* under field conditions. Besides that, terbufos and carbofuran applied at planting and application of carbofuran at post-planting also significantly reduced *P. scribneri* population in soil and also roots (Todd and Oakley 1995).

Although *Pratylenchus* spp. is satisfactorily controlled by the applications of nematicides (Olthof 1989; Philis 1997; Kimpinski, Arsenault et al. 2001), increasing concerns about the environment, food safety and public health are leading to a gradual ban or scheduled for phase-out of most of the currently used nematicides (ANON 1992; McKenry, Buzo et al. 1994). Nevertheless, nematicides are still needed in corn production due to the economic losses.

New nematicides used in seed treatment are more efficient and environmentally friendly tools for nematode management. Furthermore, coating of seeds with nematicides for commercial crop production could be the least expensive and easiest method of nematicide application. Indeed, it is the least contaminating for the environment. For example,

McGarvey (1982) suggested a potential benefit of seed coating using oxamyl to protect plants from *P. penetrans* and *Meloidogyne hapla* Chitwood in greenhouse studies and also enhanced plant growth. For example, with the advent of products such as abamectin (avermectin B<sub>1</sub>, Avicta, Syngenta Crop Protection, Inc.), N-Hibit (harpin protein, Plant Health Care, Inc.), Thiodicarb (Aeris, Bayer Crop Science, Inc.), and Votivo (*Bacillus firmus*, Bayer Crop Science, Inc.) bionematicide as a seed treatments used in cotton, soybean and corn, more options are available to control nematodes using less chemical inputs than large scale field nematicide applications.

Based on recent work (Cabrera, Kiewnick et al. 2009), abamectin proved to be very effective in reducing lesion and cyst nematodes in early infection of maize and sugar beet roots and also gall formation by root-knot nematodes in cotton. In fact this study showed that penetration of *Pratylenchus zae* was significantly reduced more than 80% in maize using a dose of 1.0 mg a.i. seed<sup>-1</sup>.

Moreover, (Monfort, Kirkpatrick et al. 2006) working with nematicidal seed treatment against *Meloidogyne incognita* on cotton, described that root galling was less severe on plants from all abamectin seed treatments than from nontreated seed, except for the 10g/100 kg of seed rate. Further, nematode reproduction was lower for all abamectin seed treatments. In contrast, in field conditions abamectin did not show the same results for infection and nematode reproduction.

For this reason assessment of the effects of those new products on nematode-fungus interactions is needed. Nematicidal seed treatments also provide new research tools to facilitate better understanding of the mechanisms of nematode-fungus interactions.

## **Fungus-nematode interactions**

Fungi play an important role in disease etiology of several diseases caused by a fungus-nematode complex. The combination of nematode and fungus often results in a synergistic interaction where in the crop loss is greater than expected from either pathogen alone or an additive effect of the two together.

Associations between nematodes and fungi results in three different types of synergism that can result in plant damage (Back, Haydock et al. 2002). It can be summarized as being positive when an association between nematode and pathogen resulting in plant damage exceeding the sum of individual damage by both pathogens ( $1 + 1 > 2$ ); Antagonistic when an association between nematode and fungus result in plant damage less than that expected from the sum of the individual pathogens ( $1 + 1 < 2$ ); and neutral when nematodes and fungi cause plant damage that equates to the sum of individual damage by both pathogens ( $1 + 1 = 2$ ).

The first report of interaction between a plant-parasitic nematode and a soil-borne plant pathogenic fungus was reported by Atkinson (1892). He showed that *Fusarium* wilt of cotton (caused by *Fusarium oxysporum* f. sp. *vasinfectum*) was more severe in soil co-infested with root-knot nematodes (*Meloidogyne* sp.). Further evidence for the interaction between *Fusarium* spp. and root-knot nematodes in cotton was later provided during field experiments in which ethylene dibromide or 1,3 dichloropropene was used to sterilize soil (Smith 1948; Newson and Martin 1953). After that, interactions of plant-parasitic nematodes with a variety of microorganisms, such as bacteria, viruses and other plant-parasitic nematodes have been described. In fact, a large volume of research documents interactions between soil-borne plant pathogens and plant-parasitic nematodes (Power 1963; Pitcher

1965; Bergeson 1972; Hirano 1975; Norton 1978; Taylor 1979; Webster 1985; Mai and Abawi 1987). .

Although many reports have been published concerning the interactions mentioned above, interactions between nematodes and fungi on maize have received little attention and little has been reported on their influence on disease interactions in maize (Palmer, MacDonald et al. 1967; Palmer and MacDonald 1974; Roth and Boothroyd 1976). Palmer and MacDonald (1974) evaluated the interaction of *Fusarium* spp. and certain plant parasitic nematodes on maize such as, *Meloidogyne incognita* and *Pratylenchus penetrans* and reported that average dry root and shoot weight of maize seedlings were significant less when both *M. incognita* and *F. moniliforme* were present than those of seedlings inoculated with either organism alone. Similar work by Palmer, MacDonald et al. (1967), also showed a synergetic interaction between *P. scribneri* and *Fusarium moniliforme* affecting fresh weight of corn than when either organism is present alone.

As mentioned before, feeding and migration of the *P. penetrans* causes considerable damage to root tissue and necrotic lesions appear on the root surface. *P. penetrans* often damage apical meristems due to the feeding process using its stylet to create a path. As a result more lateral root can develop. Consequently, wounding by nematode penetration has been assumed for many years the cause of predisposition of plants to attack by other organisms. In fact in some disease complexes nematodes can increase susceptibility to a subsequent disease (Powell 1971). In support of that concept, (Inagaki and Powell 1969) showed that mechanically wounding tobacco roots allowed symptoms of black shank infection to develop as quickly as when plants were inoculated with *Pratylenchus brachyurus*.

However, it should be pointed out that simple physical injury of host roots cannot explain all nematode-fungal interaction in many important complex diseases. For example, *Fusarium* wilt was more severe when *Meloidogyne incognita* inoculation preceded fungal inoculation of host roots by 4 weeks than when hosts were simultaneously inoculated with both pathogens (Power 1963).

*P. penetrans* interactions with fungi deserve more research, mainly because of the potential that can be derived from such work in order to better understand the mechanisms underlying these interactions.

Regarding interactions between *Pratylenchus* spp. and pathogenic fungi, probably the most frequently reported is related with wilt fungi in the genera *Fusarium* and *Verticillium* (Rowe, Riedel et al. 1985; Summer and Minton 1987). Several reports have described interactions between *Pratylenchus* spp. and several *formae speciales* of *Fusarium oxysporum* in many crops (Summer and Minton 1987). According to these studies, infection by *Pratylenchus* spp. increased the incidence or severity of *Fusarium* wilt on susceptible cultivars. Therefore, modification of *Fusarium* wilt incidence or severity may be related to the specific nematode-fungus combination.

It has been shown that *Pratylenchus* spp. appear to be the dominant nematodes involved in interaction with *Verticillium* wilt fungi. For instance, the first work with *Verticillium* wilt-nematode complexes involved primarily cotton, tomato, eggplant, and pepper (Mountain and McKeen 1962; Olthof and Reynes 1969). According to these results, either pathogen was capable of causing disease, but the damage was much greater when both were present together. According to (Bergeson 1963) and (Faulkner, Bolander et al. 1970), the incubation period of *Verticillium* wilt is shortened in *Pratylenchus* infected plants,

indicating that physical and physiological changes in the plants infected with *Pratylenchus* are related to enhanced susceptibility of plants to *Verticillium* wilt.

Hasan (1988) reported that the amounts of rotting caused in chrysanthemum roots by *Pythium aphanidermatum* and *Rhizoctonia solani* were increased in the presence of *Pratylenchus coffeae*, and were further increased when plants were attacked by all three organisms. In contrast, nematode reproduction was decreased when *Pythium aphanidermatum* was present and increased in the presence of *R. solani*, and when both were present nematode reproduction was essentially unaffected. Moreover, Jordaan, Loots et al. (1987) reported that a combination of *Pratylenchus brachyurus* and *Pratylenchus zaeae* can interact with the root-rot fungus, *Fusarium moniliforme*, on maize and this interaction can cause more severe effects on plant growth than from nematodes or fungus alone. In addition, Black root-rot of strawberries, caused by *Rhizoctonia* spp., was also more severe in the presence of *Pratylenchus penetrans*, (LaMondia and Martin 1989).

Another example of synergism between soilborne fungi and *Pratylenchus* spp. is related with potato with the interaction between *Verticillium dahliae*, that is the primary causal agent of a vascular wilt disease in potato called potato early dying, and *Pratylenchus* spp. (Rowe and Powelson 2002). Studies have shown that *Verticillium dahliae* and *P. penetrans* can interact synergistically and together they can cause more severe symptoms and reduce yields and tuber quality.

It has been reported that *Pratylenchus penetrans* is one of the most important root lesion nematodes that can enhance the development of wilt symptoms (Riedel, Rowe et al. 1985). Working with *P. penetrans* on Russet Burbank potato, with and without *V. dahliae*, (Saeed, MacQuidwin et al. 1998), demonstrated that *P. penetrans* reduce shoot weight and

photosynthesis when *V. dahliae* is present. On the other hand, in the absence of *V. dahliae*, *P. penetrans* did not reduce plant growth and tuber yield.

In contrast, based on (Martin, Riedel et al. 1982) work, 15, 50 and 150 *P. penetrans* per 100 cm<sup>3</sup> soil in combination with *V. dahliae* could result in 36, 30 and 75% yield less in potato tuber weight. Even though the mechanisms operating in these interactions are largely unknown, (Saeed, MacGuidwin et al. 1997) working with gas exchange in Russet Burbank potato, found a significant interaction between *P. penetrans* and *V. dahliae*, affecting photosynthesis and also transpiration on plants. Moreover, analogous results were showed by (Rotenberg, MacGuidwin et al. 2004), reporting that transpiration in plants that were infected with *P. penetrans* and *V. dahliae* was significantly reduced, although the combined effect was synergistic in one experiment and additive in the other.

### **Research objectives**

The objectives of this study were to measure interactions between *Pratylenchus penetrans* and fungal/oomycete pathogens (*Fusarium graminearum*, *Fusarium verticillioides*, *Pythium ultimum* and *Rhizoctonia solani*) causing seedling diseases in maize; assess the impact of nematode control with abamectin on these interactions and evaluate added benefit of abamectin combined with fungicide seed treatment for seedling disease management.

### **Literature cited**

- Acosta, N. and R. B. Malek (1979). Influence of temperature on population development of eight species of *Pratylenchus* on soybean. *Nematology* 11: 229-232.
- ANON (1992). Synthesis report of the methyl bromide interim scientific assessment and methyl bromide interim technology and economic assessment. Montreal Protocol Assessment Supplement, Nairobi, Kenya, UNEP: 33.

- Atkinson, G. F. (1892). Some diseases of cotton." Alabama Polytechnical Institute of Agriculture – Experimental Station Bulletin 41: 61-65.
- Back, M. A., P. P. J. Haydock, et al. (2002). Disease complexes involving plant parasitic nematodes and soilborne pathogens. *Plant Pathology* 51: 683-697.
- Bergeson, G. B. (1963). Influence of *Pratylenchus penetrans* alone and in combination with *Verticillium albo-atrum* on growth of peppermint. *Phytopathology* 53: 1164-1166.
- Bergeson, G. B. (1972). Concepts of nematode-fungus associations in plant disease complexes: a review. *Exp. Parasitol* 32: 301-314.
- Burpee, L. L. and J. R. Bloom (1978). The influence of *Pratylenchus penetrans* on the incidence and severity of *Verticillium* wilt of potato. *Nematology* 10: 95-99.
- Cabrera, J. A., S. Kiewnick, et al. (2009). Efficacy of abamectin seed treatment on *Pratylenchus zaeae*, *Meloidogyne incognita* and *Heterodera schachtii*. *Plant Disease and Protection* 116: 124-128.
- Castillo, P. and N. Vovlas (2007). *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. *Nematology Monographs and perspectives* 6: 529.
- Castillo, P., N. Vovlas, et al. (1998). Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. *Plant Pathology* 47: 370-376.
- Corbett, D. C. M. (1973). *Pratylenchus penetrans*. C.H.I. Description of plant-parasitic nematodes 2: 25.
- Corbett, D. C. M. (1974). *Pratylenchus penetrans*. CIH Descriptions of plant-parasitic nematodes 25.

- Dickerson, O. J., H. M. Darling, et al. (1964). Pathogenicity and population trends of *Pratylenchus penetrans* on potato and corn. *Phytopathology* 54: 317-322.
- Duncan, L. W. and J. W. Noling (1998). Agricultural sustainability and nematode integrated pest management. *American Society of Agronomy* 36: 251-287.
- Elliott, A. P. and G. W. Bird (1985). Pathogenicity of *Pratylenchus penetrans* to navy bean (*Phaseolus vulgaris L.*). *Nematology* 17: 81-85.
- Endo, B. Y. (1975). Pathogenesis of nematode-infected plants. *Annu. Rev. Phytopathol* 13: 213-238.
- Faulkner, L. R., W. J. Bolander, et al. (1970). Interactions of *Verticillium dahlia* and *Pratylenchus minyus* in *Verticillium* wilt of peppermint: Influence of the nematode as determined by a double root technique. *Phytopathology* 60: 100-103.
- Florini, D. A., R. Loria, et al. (1987). Influence of edaphic factors and previous crop on *Pratylenchus* spp. population densities in potato. *Journal of Nematology* 19: 85-92.
- Hasan, A. (1988). Interaction between *Pratylenchus coffeae* and *Pythium aphanidermatum* and/or *Rhizoctonia solani* on *chrysanthemum*. *Phytopathology* 123: 227-232.
- Hirano, K. (1975). Interrelationships between plant parasitic nematodes and other plant pathogenic organisms. *Rev. Plant. Prot. Res* 8: 55-68.
- Huan, J., G. S. Santo, et al. (1988). Effect of *Pratylenchus pentrans*, *Erwinia carotovora* subsp. *Carotovora* and *Verticillium dahliae* on potato yield in microplots. *Nematology* 20: 640-641.
- Hung, C. L. and R. A. Rohde (1973). Phenol accumulation related to resistance in tomato to infection by root-knot and lesion nematodes. *Nematology* 5: 253-258.

- Inagaki, H. and N. T. Powell (1969). Influence of the root-lesion nematode on black shank symptom development in flue-cured tobacco. *Phytopathology* 59: 1350-1355.
- Jordaan, E. M., G. C. Loots, et al. (1987). Effects of root-lesion nematodes (*Pratylenchus branchyurus* Godfrey and *P. zaeae* Graham) and *Fusarium moniliforme* Sheldon alone or in combination, on maize. *Nematologica* 33: 213-219.
- Kable, P. F. and W. F. Mai (1968). Influence of soil moisture on *Pratylenchus penetrans*. *Nematologica* 14: 101-122.
- Kimpinski, J., W. J. Arsenault, et al. (2001). Differential effect of nematicide treatments on tuber yields in early and late-maturing potato cultivars. *Plant Pathology* 50: 509-514.
- Krusberg, L. R. (1960). Hydrolytic and respiratory enzymes of species of *Ditylenchus* and *Pratylenchus*. *Phytopathology* 50: 9-22.
- LaMondia, J. A. and S. B. Martin (1989). The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. *Plant Disease* 73: 107-110.
- Loof, P. A. A. (1991). The family Pratylenchidae. In "Manual of Agricultural Nematology" (W.R.Nickle, ed.): 336-421.
- Loof, P. A. A. (1991). The family *Pratylenchidae* Thorne. *Manual of Agricultural Nematology*: 363-421.
- Mai, W. F. and G. S. Abawi (1987). Interactions among root-knot nematodes and *Fusarium* Wilt fungus on host plants. *Annu. Rev. Phytopathol* 25: 317-338.
- Mai, W. F., J. R. Bloom, et al. (1977). Biology and ecology of the parasitic nematode *Pratylenchus penetrans*. Pennsylvania State University, University Park, PA. College of Agriculture Bulletin 815: 68.

- Martin, M. J., R. M. Riedel, et al. (1982). *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72: 640-644.
- McGarvey, B. D. (1982). Studies on oxamyl: analytical method development and investigation of fate in peach seedlings and corn seeds. M.Sc. Thesis, Brock University, St. Catharines, Canada: 1-120.
- McKenry, M., T. Buzo, et al. (1994). Soil fumigants provide multiple benefits: Alternatives give mixed results. *Calif. Agric* 48: 22-28.
- Monfort, W. S., T. L. Kirkpatrick, et al. (2006). Efficacy of a novel nematicidal seed treatment against *Meloidogyne incognita* on cotton. *Journal of Nematology* 38: 245-249.
- Morgan, G. T. and A. A. MacLean (1968). Influence of soil pH on an introduced population of *Pratylenchus penetrans*. *Nematologica* 14: 311-312.
- Mountain, W. B. and C. D. McKeen (1962). Effect of *Verticillium dahliae* on the population of *Pratylenchus penetrans*. *Nematologica* 7: 261-266.
- Mountain, W. B. and Z. A. Patrick (1959). The peach replant problem in Ontario. *Can J Bot* 37: 459-470.
- Newson, L. D. and W. J. Martin (1953). Effects of soil fumigation on populations of plant parasitic nematodes, incidence of *Fusarium* wilt and yield of cotton. *Phytopathology* 43: 292-293.
- Norton, D. C. (1978). *Ecology of Plant-parasitic nematodes*. Wiley, New York, NY: 268.
- Norton, D. C. (1983). Maize nematode problems. *Plant Disease* 67: 253-256.

- Norton, D. C. (1984). Nematode parasites of corn. Plant and insect nematodes, W.R. Nickle (ed), Marcel Dekker, New York: 61-94.
- Norton, D. C. and P. N. Hinz (1976). Relationship of *Hoplolaimus galeatus* and *Pratylenchus hexincisus* to reduction of corn yields in sandy soils in Iowa. Plant Disease Reporter 60(197-200).
- Olthof, T. H. A. (1989). Effects of fumigant and nonfumigant nematicides on *Pratylenchus penetrans* and yield of potato. Nematology 21: 645-649.
- Olthof, T. H. A. and J. W. Potter (1973). The relationship between population densities of *Pratylenchus penetrans* and crop losses in summer-maturing vegetables in Ontario. Phytopathology 63: 577-582.
- Olthof, T. H. A. and J. W. Potter (1973). The relationship between populations densities of *Pratylenchus penetrans* and crop losses in summer-maturing vegetables in Ontario. Phytopathology 63: 577-582.
- Olthof, T. H. A. and A. A. Reynes (1969). Effect of *Pratylenchus penetrans* on *Verticillium* wilt of pepper (Abstr.). Nematologica 1: 21-22.
- Osteen, C. D., L. J. Moffitt, et al. (1988). Risk efficient action thresholds for nematode management. Journal of Production Agriculture 1: 332-338.
- Palmer, L. T. and D. H. MacDonald (1974). Interactions of *Fusarium* spp. and certain plant parasitic nematodes on maize. Phytopathology 64: 14-17.
- Palmer, L. T., D. H. MacDonald, et al. (1967). The ecological relationship of *Fusarium moniliforme* to *Pratylenchus scribneri* in seed blight on corn (Abstr.). Phytopathology 57: 825.

- Philis, J. (1997). Effect of cadusafos and carbofuran against *Pratylenchus penetrans* and some ectoparasitic nematodes infesting potato in Cyprus. *Nematol. Mediterr* 25: 169-172.
- Pitcher, R. S. (1965). Interrelationships of nematodes and other pathogens of plants. *Helmilthol. Abstr.* 34: 1-17.
- Pitcher, R. S., Z. A. Patrick, et al. (1960). Studies on the host-parasite relations of *Pratylenchus penetrans* (Cobb) to apple seedlings. *Nematologica* 5: 309-314.
- Potter, J. W. and J. L. Townshend (1973). Distribution of plant-parasitic nematodes in field crop soils of Southern and Central Ontario. *Plant Disease* 53: 39-48.
- Powell, N. T. (1971). Interactions between nematodes and fungi in disease complexes. *Annu. Rev. Phytopathol* 9: 253-274.
- Power, N. T. (1963). Symposium on interrelationships between nematodes and other agents causing plant disease. The role of plant parasitic nematodes in fungus diseases. *Phytopathology* 53: 28-35.
- Rich, J., R. J. Johnson, T., et al. (1985). Corn response to subsoiling and nematicide application. *Nematology* 17(404-407).
- Riedel, R. M., R. C. Rowe, et al. (1985). Differential interactions of *Pratylenchus crenatus*, *P. penetrans*, and *P. scribneri* with *Verticillium dahliae* in potato early dying disease. *Phytopathology* 75: 419-422.
- Rotenberg, D., A. E. MacGuidwin, et al. (2004). Interaction of spatially separated *Pratylenchus penetrans* and *Verticillium dahliae* on potato measured by impaired photosynthesis. *Plant Pathology* 53: 294-302.

- Roth, F. and C. W. Boothroyd (1976). Corn root rot is not caused by the interaction of species of *Fusarium* and *Pratylenchus*. (Abstr.) Proc. Am. Phytopathol. Soc 3: 244.
- Rowe, R. C. and M. L. Powelson (2002). Potato early dying: management challenges in a changing production environment. Plant Disease 86: 1184-1193.
- Rowe, R. C., R. M. Riedel, et al. (1985). Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75: 412-418.
- Saeed, I. A. M., A. E. MacGuidwin, et al. (1997). Synergism of *Pratylenchus penetrans* and *Verticillium dahliae* manifested by reduced gas exchange in potato. Phytopathology 87: 435-439.
- Saeed, I. A. M., A. E. MacQuidwin, et al. (1998). Effect of initial nematode population density on the interaction of *Pratylenchus penetrans* and *Verticillium dahliae* on Russet Burbank potato. Nematology 30: 100-107.
- Seinhorst, J. W. (1998). The common relation between population density and plant weight in pot and microplot experiments with various nematode plant combinations. Nematology 5: 459-468.
- Smith, A. L. (1948). Control of cotton wilt and nematodes with a soil fumigant. Phytopathology 38: 934-947.
- Summer, D. R. and N. A. Minton (1987). Interaction of Fusarium wilt and nematodes in Cobb soybean. Plant Disease 71: 20-23.
- Taylor, C. E. (1979). *Meloidogyne* interrelationships with microorganisms. In: F. Lamberti and C.E. Taylor (Editors), Root-Knot nematodes (*Meloidogyne* species) Systemics, Biology and Control. Academic Press, New York, NY: 375-398.

- Thistlethwayte, B. (1970). Reproduction of *Pratylenchus penetrans* (Nematoda: Tylenchida). *Nematology* 2: 101-105.
- Todd, T. C. and T. R. Oakley (1995). Evaluation of nematicides for lesion nematode control in corn. *Fungicide and Nematicide Tests* 50 (188).
- Townshend, J. L. (1963a). The pathogenicity of *Pratylenchus penetrans* to celery. *Can J Plant Sci* 43: 70-74.
- Townshend, J. L. (1963b). The pathogenicity of *Pratylenchus penetrans* to strawberry. *Can J Plant Sci* 43: 75-78.
- Townshend, J. L. (1978). Infectivity of *Pratylenchus penetrans* on alfalfa. *Nematology* 10: 318-323.
- Townshend, J. L. and L. Stobbs (1981). Histopathology and histochemistry of lesions caused by *Pratylenchus penetrans* in roots of forage legumes. *Canadian Journal of Plant Pathology* 3: 123-128.
- Troll, J. and R. A. Rohde (1966). Pathogenicity of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* on turfgrasses. *Phytopathology* 56: 995-998.
- Turner, D. R. and R. A. Chapman (1972). Infection of seedlings of alfalfa and red clover by concomitant population of *Meloidogyne incognita* and *Pratylenchus penetrans*. *Nematology* 4: 280-286.
- Uehara, T., A. Kushida, et al. (2001). PCR-based cloning of two beta-1,4-endoglucanases from the root-lesion nematode *Pratylenchus penetrans*. *Nematology* 3: 335-341.
- W.K., M. (1993). Nematode interactions. *Plant Pathology and Plant Nematology Laboratories*. Department of Botany, Aligarh Muslim University, Aligarh, India: 377.

Wallace, H. R. (1973). Nematode ecology and plant disease. London, UK, Edward Arnold:

228 pp.

Webster, J. M. (1985). Interaction of *Meloidogyne* with fungi on crop plants. J.N. Sasser and

C.C. Carter (Editors), Advanced treatise on *Meloidogyne* 1: 183-192.

Willis, C. B. (1972). Effects of soil pH on reproduction of *Pratylenchus penetrans* and forage

yield of alfalfa. Journal of Nematology 4: 291-295.

Windham, G. L. (1998). Corn. Plant and Nematode Interactions, Agronomy Monograph.

American Society of Agronomy, Madison, WI 36: 335-357.

Zunke, U. (1990). Ectoparasitic feeding behavior of the root lesion nematode, *Pratylenchus*

*penetrans*, on root hairs of different host plants. Revue Nematol 13: 331-337.

## CHAPTER 2

### **ROOT-LESION NEMATODE INTERACTIONS WITH *FUSARIUM* *GRAMINEARUM* AND *FUSARIUM VERTICILLIOIDES* ON MAIZE SEEDLINGS**

M.P. da Silva and G. P. Munkvold. Department of Plant Pathology. Iowa State University.  
Ames, IA 50011

Corresponding author: G.P. Munkvold. [munkvold@iastate.edu](mailto:munkvold@iastate.edu)

#### **Abstract**

Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites of a variety of hosts, including maize, worldwide. The mechanism of pathogenesis of *P. penetrans* is through direct feeding on roots or through interactions with other organisms in disease complexes such as those involving fungi. Lesion nematodes are known to interact with *Fusarium* pathogens in other crops, but this has not been studied extensively in maize. The objectives of this study were to measure interactions between *Pratylenchus penetrans* and *Fusarium* spp. causing seedling disease symptoms on maize; assess the impact of nematode control with abamectin on these interactions and evaluate added benefits of abamectin combined with fungicide seed treatment for seedling disease management. Growth-chamber experiments were conducted in 150 ml pots filled with an autoclaved sand-soil mixture combined with fungal inoculum of *Fusarium graminearum* or *Fusarium verticillioides* (colonized corn meal/sand mixture). A suspension of 4000 *P. penetrans* (adults and juveniles) was added to the pots at the time of planting. A factorial experimental design was

used including 8 seed treatments x 4 pathogen combinations x 6 replicates. Four replicates were harvested 30 days after planting. Shoot lengths, fresh and dry shoot and root weights, and visual root health scores were determined. Digital images of the root systems were recorded with a flatbed scanner and image analysis conducted with WinRhizo software (Regent Instruments Inc.); root length, volume, tips, branching, surface area, discoloration and diameter class distribution were determined. The remaining two replicates were harvested 42 days after planting and nematodes from soil and roots were extracted and counted. There were significant effects on root health with interactions between *Fusarium* spp. and lesion nematodes. Seed treatments showed efficacy against fungal and nematode inoculation, improving most measures of seedling health compared to the nontreated control; mainly those seed treatment combinations that included abamectin and thiabendazole. Root structure analysis from WinRhizo showed that seed treatment significantly improved root system characteristics such as root volume, root length, number of tips, forks, surface area, fine roots and diseased root length and diseased root volume. Fungal inoculation had a stronger effect compared to nematode inoculation, although, diseased root length and diseased root volume were significantly affected by nematode inoculation. Seed treatment combinations with abamectin significantly reduced diseased root length and volume when compared to the non-treated check. Abamectin in combination with seed treatment fungicides significantly reduced infection of maize root systems by lesion nematodes. Overall, seed treatments with abamectin in combination with fungicides provided the best control of seedling disease symptoms and also nematode feeding.

## Introduction

Root-lesion nematodes (*Pratylenchus* spp.) are migratory endoparasites of a variety of hosts worldwide. They can be found in all agricultural regions of the world and cause severe yield damage to the crops due the feeding on the root tissues (W.K. 1993; Castillo and Vovlas 2007). There are several species of root-lesion nematodes that can be associated with maize in Iowa, but the most economically important ones are *P. hexincisus*, *P. penetrans* and *P. scribneri* (Norton 1983; Norton 1984; Windham 1998).

*Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven (Burpee and Bloom 1978; Martin, Riedel et al. 1982; Rowe, Riedel et al. 1985; Huan, Santo et al. 1988), is one of the most common *Pratylenchus* species, with a wide range of hosts around the world and a cosmopolitan distribution throughout temperate regions (Corbett 1973; Mai, Bloom et al. 1977; Loof 1991). *Pratylenchus penetrans* is probably one of the most common species found on maize in the United States (Norton 1984) and on sweet corn in Eastern Canada (Potter and Townshend 1973). Damage caused by *P. penetrans* can easily be overlooked or mistaken because the above ground symptoms are non-specific and difficult to distinguish from other factors, such as poor fertility of the soil, lack of moisture, weather, soil pathogens or some other causes.

*Pratylenchus penetrans* juveniles and adults can enter and migrate within roots and feed on various tissues, resulting in necrotic lesions on the root surface. As a result, infections can appear along the entire root length of the host plant (Townshend and Stobbs 1981; Castillo, Vovlas et al. 1998); root lesions can fuse and become discolored with time, although the color of the lesion varies with the host (Townshend and Stobbs 1981).

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* process of pathogenicity can result in predisposition to other pathogens by wounding of the roots. Fungi play an important role in etiology of several diseases involving a nematode complex. The combination of nematode and fungus often results in a synergistic interaction wherein the crop loss is greater than expected from either pathogen alone or an additive effect of the two together (Khan, 1993).

Maize seed and seedlings are susceptible to infection by several species in the genus *Fusarium*, such as *Fusarium graminearum* and *Fusarium verticillioides* that can cause seedling diseases. The symptoms are very similar among these pathogens, such as wilting, chlorosis/yellowing, root rot and poor root development, slow growth and stunting, and post-emergence damping-off. Furthermore, maize germinates well at soil temperature above 20° C, however, symptoms can be more severe when soil temperatures are below 13° C, because germination is greatly retarded in this temperature range. In addition, seedling diseases can reduce plant population at the level that replanting sometimes is necessary (Stack 2000; Vincelli 2008; Munkvold and Robertson 2009).

The first report of an interaction between a plant-parasitic nematode and a soil-borne plant pathogenic fungus was published by Atkinson (1892); *Fusarium* wilt of cotton (caused by *Fusarium oxysporum* f. sp. *vasinfectum*) was more severe in soil co-infested with root-knot nematodes (*Meloidogyne* sp.). Further evidence for the interaction between *Fusarium* spp. and root-knot nematodes in cotton was later provided during field experiments in which ethylene dibromide or 1,3 dichloropropene was used to sterilize soil (Smith 1948; Newson and Martin 1953). It also has been shown that *Pratylenchus* spp. appear to be the dominant

nematodes involved in synergistic interactions with *Verticillium* wilt fungi (Mountain and McKeen 1962; Olthof and Reynes 1969; Rowe and Powelson 2002).

To control nematodes in maize, soil nematicides are still being used despite environmental concerns, and can significantly reduce nematode populations. For instance, applications of 1,3-D and carbofuran combined in Iowa resulted in good control of *P. hexincisus* (Norton and Hinz 1976). However, increasing concerns about the environment, food safety and public health are leading to a gradual ban or scheduled for phase-out of most of the currently used nematicides (ANON 1992; McKenry, Buzo et al. 1994).

Nematicides used as seed treatments are more efficient and environmentally friendly tools for nematode management, compared to soil applications. For example, with the advent of products such as Avicta (avermectin B<sub>1</sub>, , 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 nematodes using less chemical inputs than was necessary with soil applications of nematicides. For instance, recent work with abamectin proved to be very effective in reducing lesion and cyst nematodes in early infection of maize and sugar beet roots and also gall formation by root-knot nematodes in cotton (Cabrera, Kiewnick et al. 2009). In fact this study showed that penetration of *Pratylenchus zae* was significantly reduced more than 80% in maize using a dose of 1.0 mg a.i. seed<sup>-1</sup>. For this reason assessment of the effects of those new products on nematode-fungus interactions in maize is needed. Nematicidal seed treatment also provides new research tools to facilitate better understanding of the mechanisms of nematode-fungus interactions.

The objectives of this study were to measure interactions between *Pratylenchus penetrans* and *Fusarium* spp. causing seedling disease symptoms on maize, especially root system effects; assess the impact of nematode control with abamectin on these interactions; and evaluate added benefits of abamectin combined with fungicide seed treatment for seedling disease management.

### **Material and methods**

**General design:** Experiments were conducted twice in a growth chamber at the Iowa State University, Ames, Iowa. A full factorial experimental design was used. Experimental factors were seed treatment (eight treatments), nematode infestation (infested or not infested), and fungal infestation (infested or not infested). Seed of one maize hybrid (NK Brand hybrid N40T-GT, Syngenta Seeds, Golden Valley, MN) was treated with seven different combinations of active ingredients and a non-treated control was included (Table 1). Seed treatment rates used were the recommended rates for commercial use. Treatments were arranged a growth chamber as a completely randomized block design with six replicates. Each pot (cone) was an experimental unit.

**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, Plattner 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. Each bag was then inoculated by injecting 2 ml of a spore suspension ( $10^6$  conidia  $\text{ml}^{-1}$ ) of one of the *Fusarium* isolates, prepared from cultures on carnation leaf agar (CLA) (Leslie and Summerell 2006). The bags were then

incubated in the dark at ambient temperature (20 to 24°C) for six days, with mixing every day. Autoclaved sand-soil (1 part soil: 2 parts sand) was mixed with fungal inoculum. The proportion was 30% of inoculum and 70% by volume of sand-soil mixture. Pots (150 ml) were filled with the mixture. A small piece of paper towel was placed in the bottom of each cone to partially retard drainage. One maize seed was placed in each cone. *Pratylenchus penetrans*, provided by Dr. A.E. MacGuidwin (University of Wisconsin, Madison), was cultured monoxenically (Layne and A.E. MacGuidwin 1994) on excised sweet corn roots in Gambor's B-5 Medium with vitamins and without cytokinins or auxin (Gamborg, Murashige 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 2000 nematodes/ml determined by nematode counting slide. Nematodes were added to the cones by injection of 2 ml suspension of nematodes (equivalent to 4000 nematodes) using a microliter pipette at the time of planting (Saeed, MacGuidwin et al. 1999).

**Growing conditions:** Plants were maintained in a growth chamber under light supplied by cool white fluorescent and incandescent lamps with a photoperiod of 14 hours. Relative humidity was maintained at 99% and temperature was 22° C ( $\pm 0.1$  C). The plants were watered once a day using a watering can (20 ml per plant) and fertilized once a week, using Peters Excell water soluble fertilizer (15-5-15).

**Data collection and analysis:** Four replicates were harvested 30 days after planting. They were removed from the cones and the roots were well washed. Shoot lengths (flag leaf),

fresh shoot and root weights were measured. Shoots and roots were oven-dried at 110°C for 24h and weighed.

To perform analyses of root color and morphology for each treatment, roots were scanned and image analyses conducted with the software WinRhizo 2008a (Regent Instruments Inc., Quebec, Canada). The procedures were as follows: washed and intact roots were spread out in a transparent tray in order to avoid overlapping during scanning process. A blue piece of plastic served as image background. Image recording was performed at a resolution of 600 dpi using a 24bit color mode, and images were saved as TIFF (tagged image file format). All other scanner settings, such as dust removal, etc. were turned off. A Dell Precision T3500 was used to drive the scanner (an Epson Perfection V700 Photo – Dual Lens System). In order to perform color analyses with the WinRhizo software, the first step was calibrating the program to distinguish the background and the healthy and diseased roots. This was done by creating color classes and groups, the latter being a set of classes which defined the range of colors assigned to each class (healthy roots, diseased roots, or background). In addition to the color analyses, root morphology also was determined and the following measurements were recorded: total root length (cm), total surface area (cm<sup>2</sup>), total volume (cm<sup>3</sup>), number of tips, number of forks and length (cm) of fine roots (<0.5 mm diameter).

**Nematode extraction:** Two replicates per treatment were harvested after six weeks in order to extract nematodes from soil and roots. A 100 cm<sup>3</sup> 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). Roots were cut into small pieces (1 cm) long, mixed and incubated in Baermann funnels for two days. After nematodes were collected, the

roots were dried at 100°C for two days and weighed. The number of *P. penetrans* (juveniles and adults) was counted using an inverted microscope. The total number of nematodes present per pot was calculated based on soil volume and root weight. Also the number of *P. penetrans* per gram of dry root weight was determined.

**Statistical analysis:** Data collected were analyzed by analysis of variance (ANOVA) for a completely randomized block design (SAS Inc., Cary, NC). Least significant differences ( $P \leq 0.05$ ) 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, analyses of variance were performed on transformed ( $\log_{10}$ ) data except for disease root length (%), disease root volume (%) and nematode counts. Analysis of all main effects and interactions was conducted using all treatment combinations. Because of significant seed treatment by pathogen infestation interactions, seed treatment effects also were tested for *Fusarium* spp. and *P. penetrans*-infested treatments only, excluding the non-inoculated treatments.

## Results

### *Fusarium graminearum* experiments

Fungal infestation significantly affected ( $P < 0.0001$ ) all the variables measured. Nematode infestation significantly affected shoot dry ( $P = 0.003$ ) and root dry ( $P = 0.004$ ) weights, diseased root length ( $P = 0.03$ ) and diseased root volume ( $P = 0.0008$ ). Also there were significant interactions between *F. graminearum* and *P. penetrans* ( $P = 0.009$ ) affecting root dry weights and fine roots ( $P = 0.04$ ) (Table 2). Seed treatment had significant effects on root length ( $P = 0.0002$ ), root volume ( $P = 0.01$ ), number of tips ( $P = 0.001$ ), forks ( $P = 0.0003$ ), surface area ( $P < 0.0001$ ), fine roots ( $P = 0.002$ ), disease root length ( $P = 0.03$ ) and disease root

volume ( $P=0.03$ ). There were significant interactions between seed treatment and fungal inoculation affecting root length ( $P=0.0009$ ), number of tips, ( $P=0.01$ ), forks ( $P=0.001$ ), surface area ( $P=<.0001$ ), diseased root length ( $P=0.02$ ) and diseased root volume ( $P=0.005$ ). Interactions between seed treatment and nematode inoculation just affected disease root volume ( $P=0.008$ ). There were significant effects of seed treatment on the numbers of nematodes extracted from soil ( $P<0.0001$ ) and from the roots ( $P=0.004$ ) (Tables 3 and 4).

There were no significant 3-way interactions between seed treatment, fungal infestation and nematode infestation (Table 2).

Analysis of variance for seed treatment effects on *F. graminearum*--infested and nematode- infested plants indicated significant seed treatment effects for shoot length, root fresh, root dry, root length, root volume, number of tips, number of forks, root surface area, fine roots, disease root length and disease root volume, but not for the other variables (Figures 1 to 4). Treatments 6 (FMA + Abamectin + Thiabendazole) and 7 (FMA + Abamectin + Thiamethoxam + Thiabendazole) often resulted in the highest means for the various plant health variables (Figures 2 A, B and 3 A).

### ***Fusarium verticillioides* experiments**

Fungal inoculation significantly affected ( $P<.0001$ ) root length, root volume, number of tips, number of forks, root surface area, number of fine roots, disease root length and disease root volume. At the same time, nematode inoculation significantly affected ( $P<.001$ ) root length, number of tips, number of forks, root surface area, number of fine roots, root volume ( $P=0.0001$ ), diseased root length ( $P=0.04$ ) and diseased root volume ( $P=0.03$ ). There were significant interactions between seed treatment and fungal inoculation affecting root length ( $P=0.001$ ), root volume ( $P=0.01$ ), number of tips ( $P=0.004$ ), number of forks

( $P=0.003$ ), root surface area ( $P=0.001$ ) and the number of fine roots ( $P=0.001$ ). Furthermore, there were significant interactions between fungal inoculation and nematode inoculation affecting root length ( $P=0.004$ ), number of tips ( $P=0.004$ ), number of forks ( $P=0.005$ ), root surface area ( $P=0.009$ ), number of fine roots ( $P=0.001$ ), disease root length ( $P=0.009$ ) and disease root volume ( $P=0.02$ ). The effects on the remaining variables were not significant (Table 5). There was a significant effect of seed treatment ( $P=0.0005$ ) on the numbers of *P. penetrans* extracted from soil. There was a significant effect of seed treatment ( $P=0.003$ ) and also a significant interaction between seed treatment and fungal inoculation affecting the numbers of nematodes extracted from the roots (Tables 6 and 7). Analysis of variance for seed treatment effects on *F. verticillioides*-infested plants indicated significant seed treatment effects for shoot fresh weight, shoot dry weight, root fresh and dry weights, root length, root volume, number of tips, number of forks, root surface area, number of fine roots, disease root length and disease root volume (Figures 9 B,C,D, 10,11 and 12). The analysis of variance for seed treatment on *P. penetrans*-infested plants indicated significant seed treatment effects for shoot length, shoot fresh, shoot dry, root length, root volume, number of tips, number of forks, number of fine roots, disease root length and disease root volume, but not for the other variables (Figures 13 A,B,C, 14 B,C,D, 15 A,C,D and 16).

### 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 stronger effect than nematode inoculation for most of the variables. Regarding the interactions, there was a synergistic interaction between *F. graminearum* infestation and nematode infestation affecting root dry

weight and the number of fine roots; and there were significant interactions between *F. verticillioides* and *P. penetrans* for several root health variables. Seed treatment combinations that included abamectin or abamectin with thiabendazole resulted in the healthiest and largest root systems compared to the non-treated check or to a fungicide/insecticide seed coating without the nematicide (Figures 1, 2 and 17). These results are in conformity with Monfort, Kirkpatrick et al. (2006) working with cotton, who obtained greater plant height in plants treated with abamectin than in control plants. These treatment combinations also showed efficacy reducing disease root length and disease root volume when fungi and nematodes were present (Figures 11 D, 12 A, 15 D and 16 A).

Noninoculated treatments were not affected by seed treatment according to the variables used in this study. There were no negative effects concerning phytotoxicity, such as lower germination and/or stunting plants.

Populations of *P. penetrans* from soil and roots were significantly affected by seed treatment. Combinations including abamectin had higher populations of *P. penetrans* in the soil, but lower populations in the roots, compared to treatments lacking abamectin (Table 4 and 7). Apparently, seed treatment combinations 5, 6 and 7 protected the maize roots, reducing nematode penetration. Similarly, Cabrera, Kiewnick et al. (2009), working with efficacy of abamectin seed treatment on *Pratylenchus zae*, revealed that penetration of *P. zae* was reduced more than 80% in maize at a dose of 1.0 mg a.i. seed<sup>-1</sup>. At the same time, *P. penetrans* population from roots also was significantly affected by seed treatment combination with abamectin reducing the number of nematodes in the roots. As a result, abamectin treatments have shown the higher number of nematodes recovered from soil.

Abamectin has a broad-spectrum nematicide providing protection against a wide range of parasitic nematodes, including: lesion, root-knot, stubby-root, lance, sting, dagger, needle, ring, spiral and stunt nematodes. 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.

Emergence rate 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°C to 25°C). *Fusarium* spp. can cause more severe symptoms in maize seed and seedlings when soil temperature are below 13° C, mainly because germination is greatly retarded in this temperature range. Therefore, in order to fully understand lesion nematode interactions with seedling pathogens, these studies should be repeated under a range of temperatures.

Root structure analysis from WinRhizo showed seed treatment significantly improved root system characteristics such as root volume, root length, number of tips, forks, surface area, fine roots and reduced disease root length and disease root volume. Diseased root length and diseased root volume were significantly affected by nematode infestation, and these symptoms were significantly reduced by seed treatment combinations with abamectin (Figures 3 D and 4 A).

There was significant interaction between seed treatment and nematode inoculation (STxN) affecting diseased root volume. In this particular case abamectin in combination with

(FMA + Thiamethoxan + Thiabendazole (treatment 7) significantly reduced disease root volume (Figure 8 A). 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 around the world (Kudo, N. 2007). There may be some evidence of this in our results; for *P. penetrans*-infested treatments, only the seed treatment combination with both abamectin and thiabendazole (treatment 6) was significantly different from the control for several of the variables (Figs. 5, 6, 7).

Using WinRhizo for 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, Loiskandl 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 (Eric, Richard et al. 1993; Zobel, Kinraide et al. 2007; Himmelbauer, Loiskandl et al. 2004).

Root structure data from WinRhizo indicated similar effects to fungal and nematode infestation, but effects were more dramatic with *F. graminearum* infestation of the potting medium. Furthermore, seed treatment displayed significant 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.

Comparing the two *Fusarium* species, there were some differences regarding seed treatment effects, fungal inoculation, nematode inoculation and fungal-nematode interactions (Tables 2 and 7). For instance, *Fusarium verticillioides* experiments showed greater effects

of nematode inoculation and fungal-nematode interaction. The *Fusarium 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 suggests that the more aggressive *F. graminearum* isolate was better able to infect roots without the aid of nematode wounding, 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, MacDonald et al. (1967) and Palmer and MacDonald (1974), working with interactions of *Fusarium* spp. and certain plant parasitic nematodes on maize, reported a synergistic interaction of *P. scribneri* and *F. verticillioides* affecting fresh weight on corn when both organism were present. Moreover, Jordaan, Loots et al. (1987) reported that a combination of *Pratylenchus brachyurus* and *Pratylenchus zae* can interact with the root-rot fungus, *Fusarium moniliforme* (syn. *F. verticillioides*), on maize and this interactions can cause more severe effects on plant growth than from nematodes or fungus alone. *Fusarium* isolates within a species can display considerable variability, so it is not clear whether our results regarding differences between *F. graminearum* and *F. verticillioides* would be consistent across multiple isolates of each species.

To our knowledge, this study is the first to evaluate and measure the interactions between *P. penetrans* and *Fusarium* spp. on maize roots, and the potential benefits of abamectin combined with fungicidal seed treatment in the presence of both *Fusarium* spp. and lesion nematodes on maize. Data obtained in this study provides evidence that abamectin in combination with fungicidal seed treatments significantly improved the protection of the maize root system against seedling disease symptoms. In addition to that, our research

presents novel data regarding root system characteristics in response to fungal and nematode inoculation and seed treatments, using WinRhizo (Regent Instruments Inc., Quebec, Canada)

### Literature cited

- ANON (1992). Synthesis report of the methyl bromide interim scientific assessment and methyl bromide interim technology and economic assessment. Montreal Protocol Assessment Supplement, Nairobi, Kenya, UNEP: 33.
- Atkinson, G. F. (1892). Some diseases of cotton. Alabama Polytechnical Institute of Agriculture – Experimental Station Bulletin 41: 61-65.
- Burpee, L. L. and J. R. Bloom (1978). The influence of *Pratylenchus penetrans* on the incidence and severity of *Verticillium wilt* of potato. *Nematology* 10: 95-99.
- Cabrera, J. A., S. Kiewnick, et al. (2009). Efficacy of abamectin seed treatment on *Pratylenchus zaeae*, *Meloidogyne incognita* and *Heterodera schachtii*. "Plant Disease and Protection 116: 124-128.
- Castillo, P. and N. Vovlas (2007). *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. *Nematology Monographs and perspectives* 6: 529.
- Castillo, P., N. Vovlas, et al. (1998). Pathogenicity and histopathology of *Pratylenchus thornei* populations on selected chickpea genotypes. *Plant Pathology* 47: 370-376.
- Corbett, D. C. M. (1973). *Pratylenchus penetrans*. C.H.I. Description of plant-parasitic nematodes 2: 25.
- Desjardins, A. E., R. D. Plattner, et al. (1995). Genetic analysis of fumonisin production and virulence of *Giberella fujikuroi*, mating population A (*Fusarium moniliforme*) on maize (*Zea mays*) seedlings. *Applied Environ. Microbiol* 61: 79-86.

- Endo, B. Y. (1975). Pathogenesis of nematode-infected plants. *Annu. Rev. Phytopathol* 13: 213-238.
- Eric, P., A. H. Richard, et al. (1993). Using a fine root extraction device to quantify small diameter corn roots (>0.025 mm) in field soils." *Plant and Soil* 153: 273-279.
- Gamborg, O. L., T. Murashige, et al. (1976). Plant tissue culture media. *In Vitro* 12: 473-8.
- Himmelbauer, M. L., W. Loiskandl, et al. (2004). Estimating length, average diameter and surface area of fine roots using two different image analyses systems. *Plant and Soil* 260: 111-120.
- Huan, J., G. S. Santo, et al. (1988). Effect of *Pratylenchus penetrans*, *Erwinia carotovora* subsp. *Carotovora* and *Verticillium dahliae* on potato yield in microplots. *Nematology* 20: 640-641.
- Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for extracting nematodes from soil. *Plant Disease Reporter* 48: 692.
- Jordaan, E. M., G. C. Loots, et al. (1987). Effects of root-lesion nematodes (*Pratylenchus branchyurus* Godfrey and *P. zae* Graham) and *Fusarium moniliforme* Sheldon alone or in combination, on maize. *Nematologica* 33: 213-219.
- Kimpinski, J., W. J. Arsenault, et al. (2001). Differential effect of nematicide treatments on tuber yields in early and late-maturing potato cultivars. *Plant Pathology* 50: 509-514.
- Layne, T. L. and A.E.MacGuidwin (1994). Recovery of *Pratylenchus penetrans* from plant tissue cultures. *Nematology*: 26-557 (Abstr.).
- Layne, T. L. and A. E. MacGuidwin (1994). Recovery of *Pratylenchus penetrans* from plant tissue culture. *Nematology* 26: 557 (Abstract).

- Leslie, J. F. and B. A. Summerell (2006). *The Fusarium laboratory manual*. Blackwell Publishing, Ames, IA.
- Loof, P. A. A. (1991). The family *Pratylenchidae* Thorne. *Manual of Agricultural Nematology*: 363-421.
- Mai, W. F., J. R. Bloom, et al. (1977). Biology and ecology of the parasitic nematode *Pratylenchus penetrans*. Pennsylvania State University, University Park, PA. College of Agriculture Bulletin 815: 68.
- Martin, M. J., R. M. Riedel, et al. (1982). *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72: 640-644.
- McKenry, M., T. Buzo, et al. (1994). Soil fumigants provide multiple benefits: Alternatives give mixed results. *Calif. Agric* 48: 22-28.
- Monfort, W. S., T. L. Kirkpatrick, et al. (2006). Efficacy of a novel nematicidal seed treatment against *Meloidogyne incognita* on cotton. *Journal of Nematology* 38: 245-249.
- Mountain, W. B. and C. D. McKeen (1962). Effect of *Verticillium dahliae* on the population of *Pratylenchus penetrans*. *Nematologica* 7: 261-266.
- Munkvold, G. P. and J. K. O'Mara (2002). Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedlings blight caused by *Fusarium* species. *Plant Disease* 86: 143-150.
- Newson, L. D. and W. J. Martin (1953). Effects of soil fumigation on populations of plant parasitic nematodes, incidence of *Fusarium* wilt and yield of cotton. *Phytopathology* 43: 292-3.

- Norton, D. C. (1983). Maize nematode problems. *Plant Disease* 67: 253-256.
- Norton, D. C. (1984). Nematode parasites of corn. *Plant and insect nematodes*, W.R. Nickle (ed), Marcel Dekker, New York: 61-94.
- Norton, D. C. and P. N. Hinz (1976). Relationship of *Hoplolaimus galeatus* and *Pratylenchus hexincisus* to reduction of corn yields in sandy soils in Iowa. *Plant Disease Reporter* 60 (197-200).
- Olthof, T. H. A. (1989). Effects of fumigant and nonfumigant nematicides on *Pratylenchus penetrans* and yield of potato. *Nematology* 21: 645-649.
- Olthof, T. H. A. and A. A. Reynes (1969). Effect of *Pratylenchus penetrans* on Verticillium wilt of pepper (Abstr.). *Nematologica* 1: 21-22.
- Palmer, L. T. and D. H. MacDonald (1974). Interactions of *Fusarium* spp. and certain plant parasitic nematodes on maize. *Phytopathology* 64: 14-17.
- Palmer, L. T., D. H. MacDonald, et al. (1967). The ecological relationship of *Fusarium moniliforme* to *Pratylenchus scribneri* in seed blight on corn (Abstr.). *Phytopathology* 57: 825.
- Philis, J. (1997). Effect of cadusafos and carbofuran against *Pratylenchus penetrans* and some ectoparasitic nematodes infesting potato in Cyprus. *Nematol. Mediterr* 25: 169-172.
- Potter, J. W. and J. L. Townshend (1973). Distribution of plant-parasitic nematodes in field crop soils of Southern and Central Ontario. *Plant Disease* 53: 39-48.
- Rowe, R. C. and M. L. Powelson (2002). Potato early dying: management challenges in a changing production environment. *Plant Disease* 86: 1184-1193.

- Rowe, R. C., R. M. Riedel, et al. (1985). Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. *Phytopathology* 75: 412-418.
- Saeed, I. A. M., A. E. MacGuidwin, et al. (1999). Limitation to photosynthesis in *Pratylenchus penetrans* - and *Verticillium dahliae* - infected potato. *Crop Science* 39: 1340-1346.
- Smith, A. L. (1948). Control of cotton wilt and nematodes with a soil fumigant. *Phytopathology* 38: 934-947.
- Townshend, J. L. and L. Stobbs (1981). Histopathology and histochemistry of lesions caused by *Pratylenchus penetrans* in roots of forage legumes. *Canadian Journal of Plant Pathology* 3: 123-128.
- W.K., M. (1993). Nematode interactions. *Plant Pathology and Plant Nematology Laboratories*. Department of Botany, Aligarh Muslim University, Aligarh, India: 377.
- Windham, G. L. (1998). Corn. *Plant and Nematode Interactions*, Agronomy Monograph. American Society of Agronomy, Madison, WI 36: 335-357.
- Zobel, R. W., T. B. Kinraide, et al. (2007). Fine root diameters can change in response in nutrient concentration. *Plant and Soil* 297(1-2): 243-254.

Table 1. Seed treatments used in the experiments. Treatment 1 is the commercial standard and Treatments 2 through 7 include fludioxonil, mefenoxam, and azoxystrobin (FMA) at the same rates as Treatment 1.

Treatment	Active ingredients	Chemical group	Formulation (%)	Brand name	Rate
1	Fludioxonil	Phenylpyrrole	40.3	Maxim	2.5g/100kg
	Mefenoxam	Phenylamide	1.1	Apron XL	2g/100kg
	Azoxystrobin	Strobilurin	9.6	Dynasty	1g/100kg
2	FMA <sup>a</sup> + Thiabendazole	Benzimidazole	42.3		20g/100kg
3	FMA + Thiamethoxam	Neonicotinoid	47.6	Cruiser	0.25mg/seed
4	FMA + Thiamethoxam + Thiabendazole	Neonicotinoid Benzimidazole	47.6 42.3	Cruiser	0.25mg/seed
	FMA + Abamectin	Avermectin	46.3	Avicta	0.25mg/seed
6	FMA + Abamectin + Thiabendazole	Avermectin Benzimidazole	12.4	Avicta	0.25mg/seed
	FMA + Abamectin + Thiamethoxam + Thiabendazole	Avermectin Neonicotinoid Benzimidazole	12.4	Avicta Cruiser	0.25mg/seed
8	Untreated		0		

<sup>a</sup> FMA = fludioxonil + mefenoxam + azoxystrobin = Treatment 1

Table 2. *P*-values for effects of seed treatments and pathogen inoculation on seedling health variables for *Fusarium graminearum* experiments. Analysis of variance was conducted with log transformed data except for disease root length (%) and disease root volume (%) which were not transformed.

Variables	Effects						
	Seed treatment (ST)	Fungus (F)	Nematode (N)	ST x F	ST x N	F x N	ST x F x N
Shoot length (cm)	ns	<.0001	ns	ns	ns	ns	ns
Shoot fresh (g)	ns	<.0001	ns	ns	ns	ns	ns
Shoot dry (g)	ns	<.0001	0.003	ns	ns	ns	ns
Root fresh (g)	ns	<.0001	ns	ns	ns	ns	ns
Root dry (g)	ns	<.0001	0.004	ns	ns	0.009	ns
Root length (cm)	0.0002	<.0001	ns	0.0009	ns	ns	ns
Root volume (cm <sup>3</sup> )	0.01	<.0001	ns	ns	ns	ns	ns
Tips	0.001	<.0001	ns	0.01	ns	ns	ns
Forks	0.0003	<.0001	ns	0.001	ns	ns	ns
Surface area (cm <sup>2</sup> )	<.0001	<.0001	ns	<.0001	ns	ns	ns
Fine roots (cm)	0.002	<.0001	ns	ns	ns	0.04	ns
Disease root length (%)	0.03	<.0001	0.03	0.02	ns	ns	ns
Disease root volume (%)	0.03	<.0001	0.0008	0.005	0.008	ns	ns

\* ns = not significant ( $p>0.05$ )

Table 3. *P*-values for effects of seed treatments and pathogen inoculation on nematode extraction results for *Fusarium graminearum* experiments.

	Effects		
	Seed treatment (ST)	Fungal inoculation (F)	ST x F
Nematodes from soil	<.0001	ns	ns
Nematodes from roots	0.004	ns	ns

\* ns = not significant ( $p>0.05$ )

Table 4. Number of *Pratylenchus penetrans* recovery from 100 cm<sup>3</sup> of soil and the roots therein and from 1 g of dried maize roots from treatment infested with the nematodes and with or without *Fusarium graminearum* infestation

Treatment	Active ingredients	Nematodes / 100 cm <sup>3</sup> soil	Nematodes / g root
1	Fludioxonil + Mefenoxam + Azoxystrobin	57.5b	17.5a
2	FMA <sup>a</sup> + Thiabendazole	60.8b	13.3a
3	FMA + Thiamethoxam	49.3b	12a
4	FMA + Thiamethoxam + Thiabendazole	62.3b	10ab
5	FMA + Abamectin	171.8a	1.8bc
6	FMA + Abamectin + Thiabendazole	156.5a	0c
7	FMA + Abamectin + Thiamethoxam + Thiabendazole	205.5a	0c
8	Untreated	81.5b	9.5ab

<sup>a</sup> FMA = fludioxonil + mefenoxam + azoxystrobin = Treatment 1

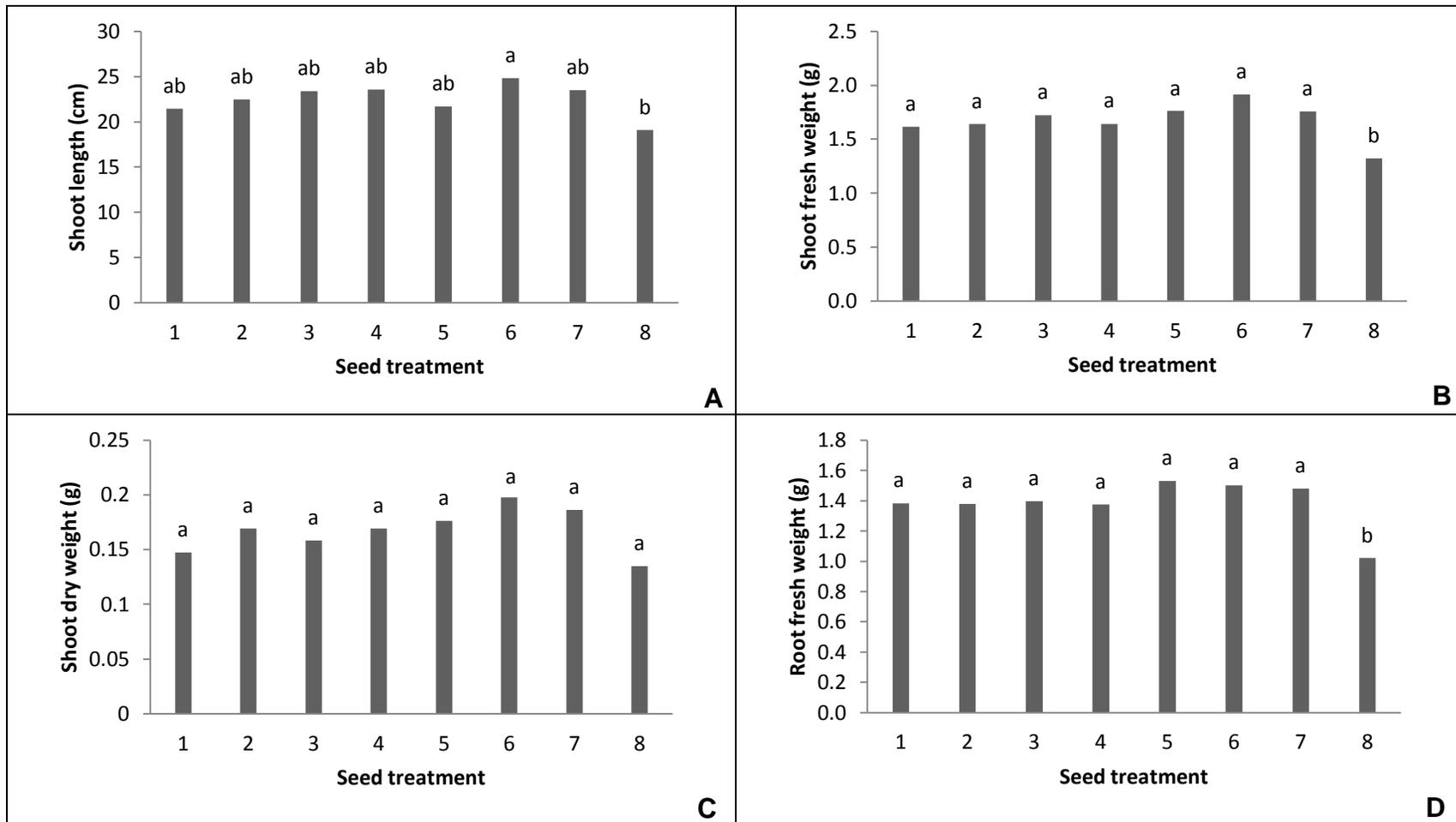


Figure 1. Shoot length means (A), shoot fresh weight means (B), shoot dry weight means (C), and root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Fusarium graminearum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiabendazole, Treatment 4 = FMA + Thiabendazole + Thiamethoxam, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

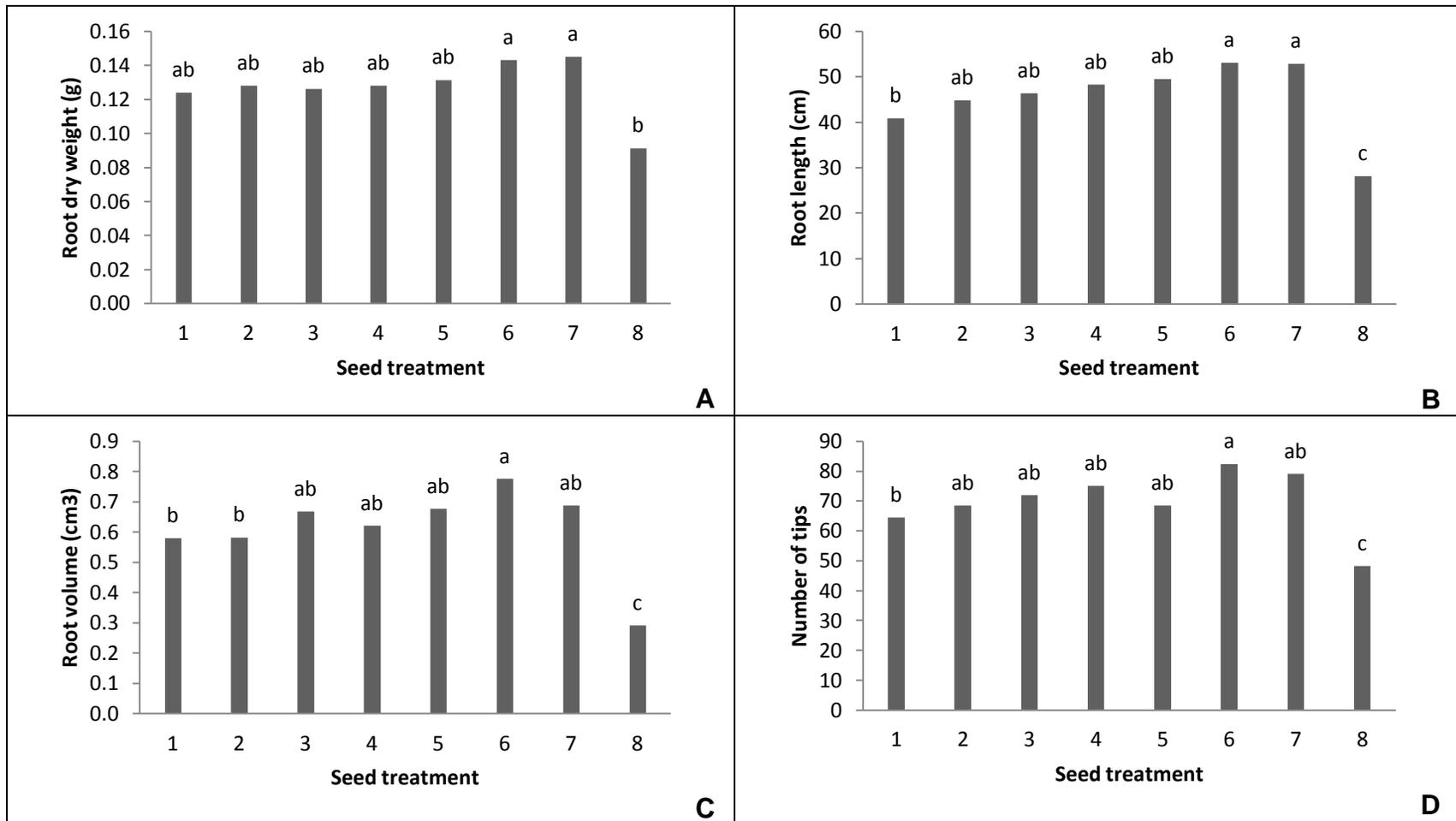


Figure 2. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Fusarium graminearum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

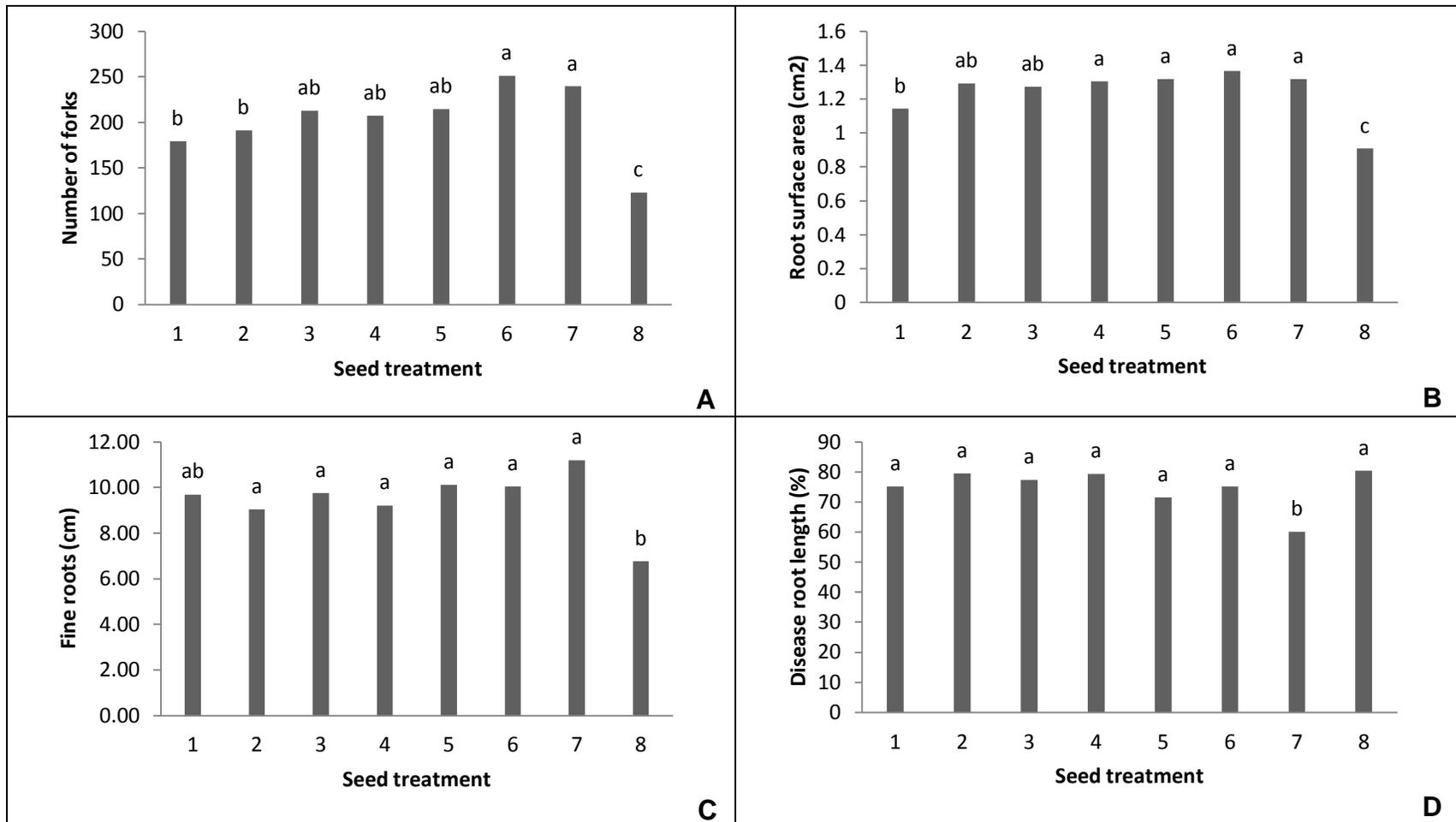


Figure 3. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Fusarium graminearum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiabendazole, Treatment 4 = FMA + Thiabendazole + Thiamethoxam, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

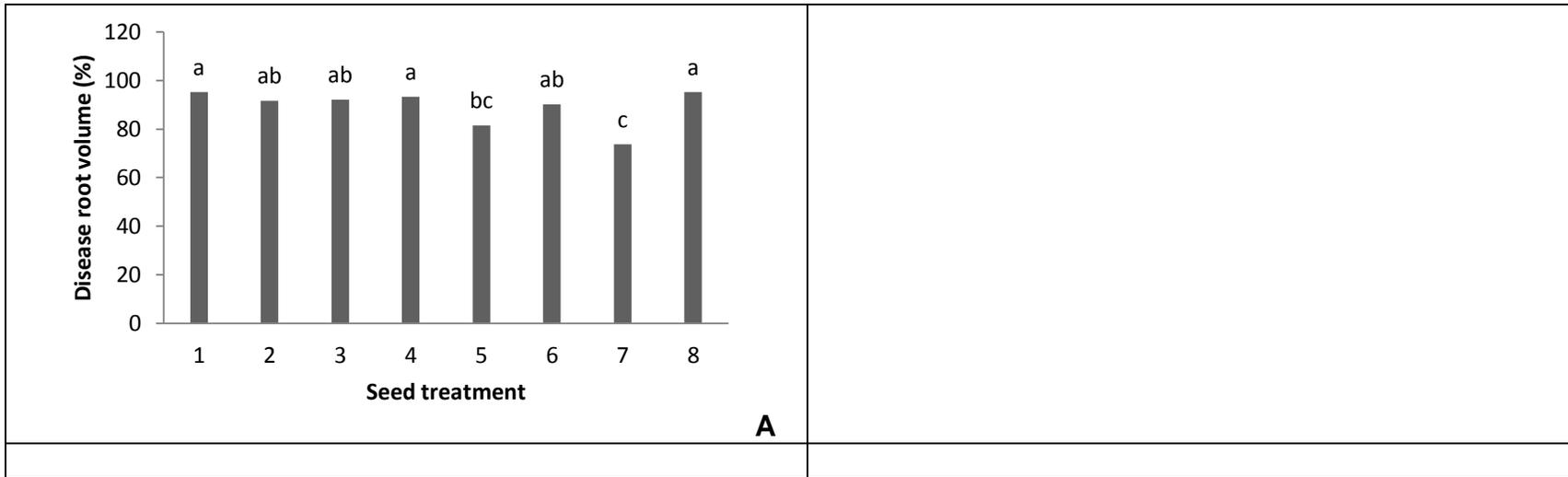


Figure 4. Disease root volume (%) for seedlings from seeds treated with different seed treatment products and grown in *Fusarium graminearum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

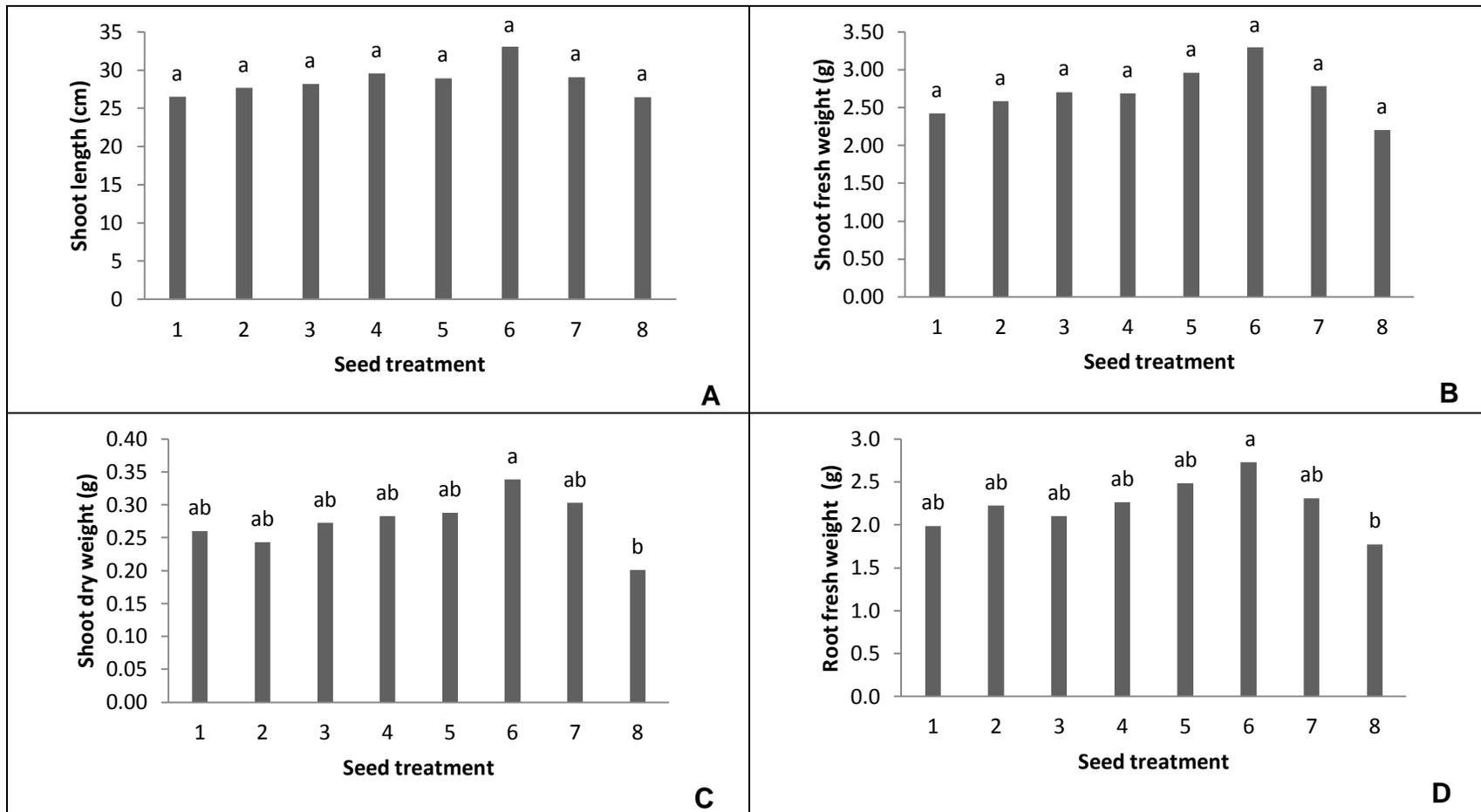


Figure 5. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium graminearum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

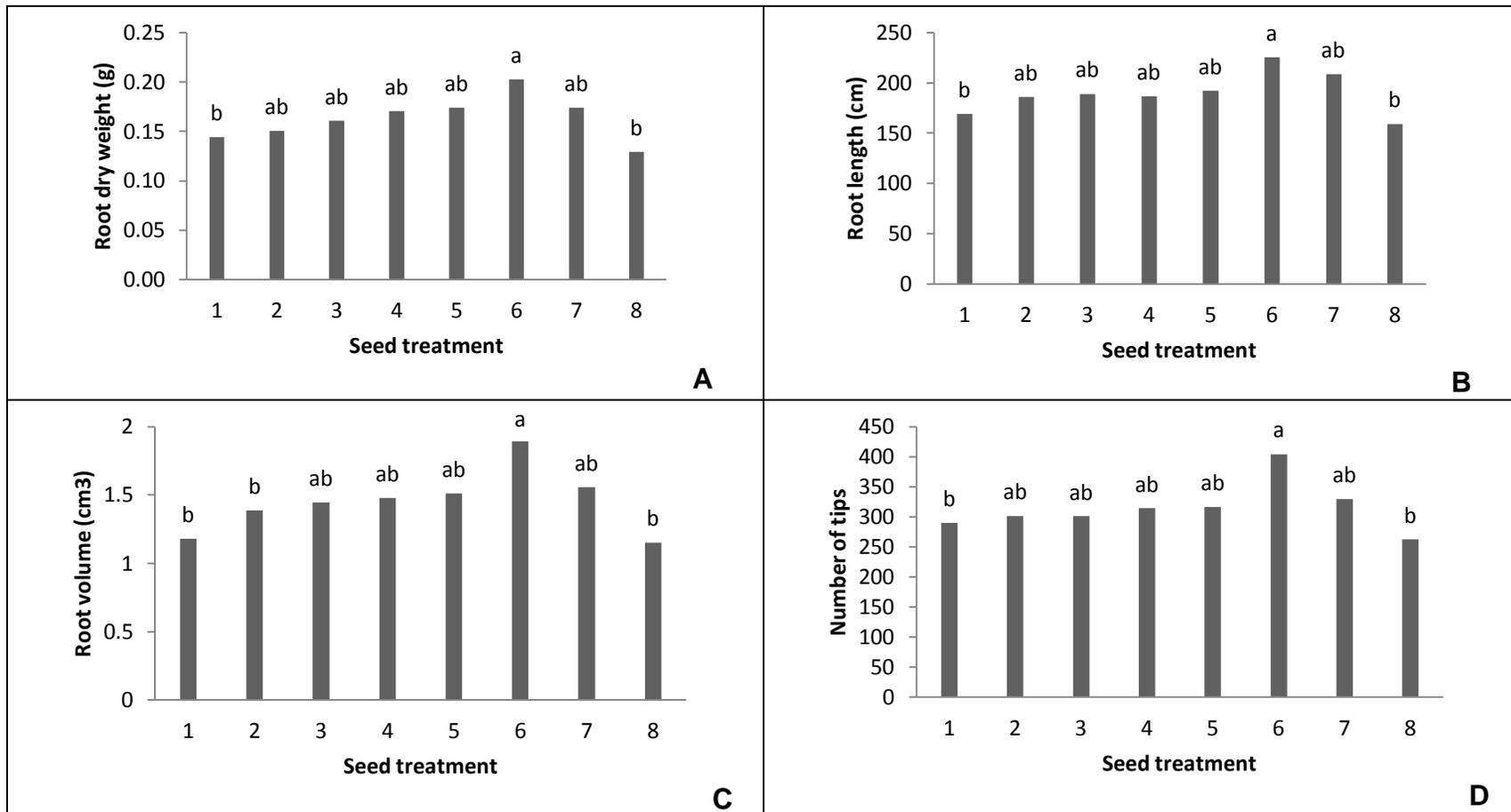


Figure 6. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium graminearum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

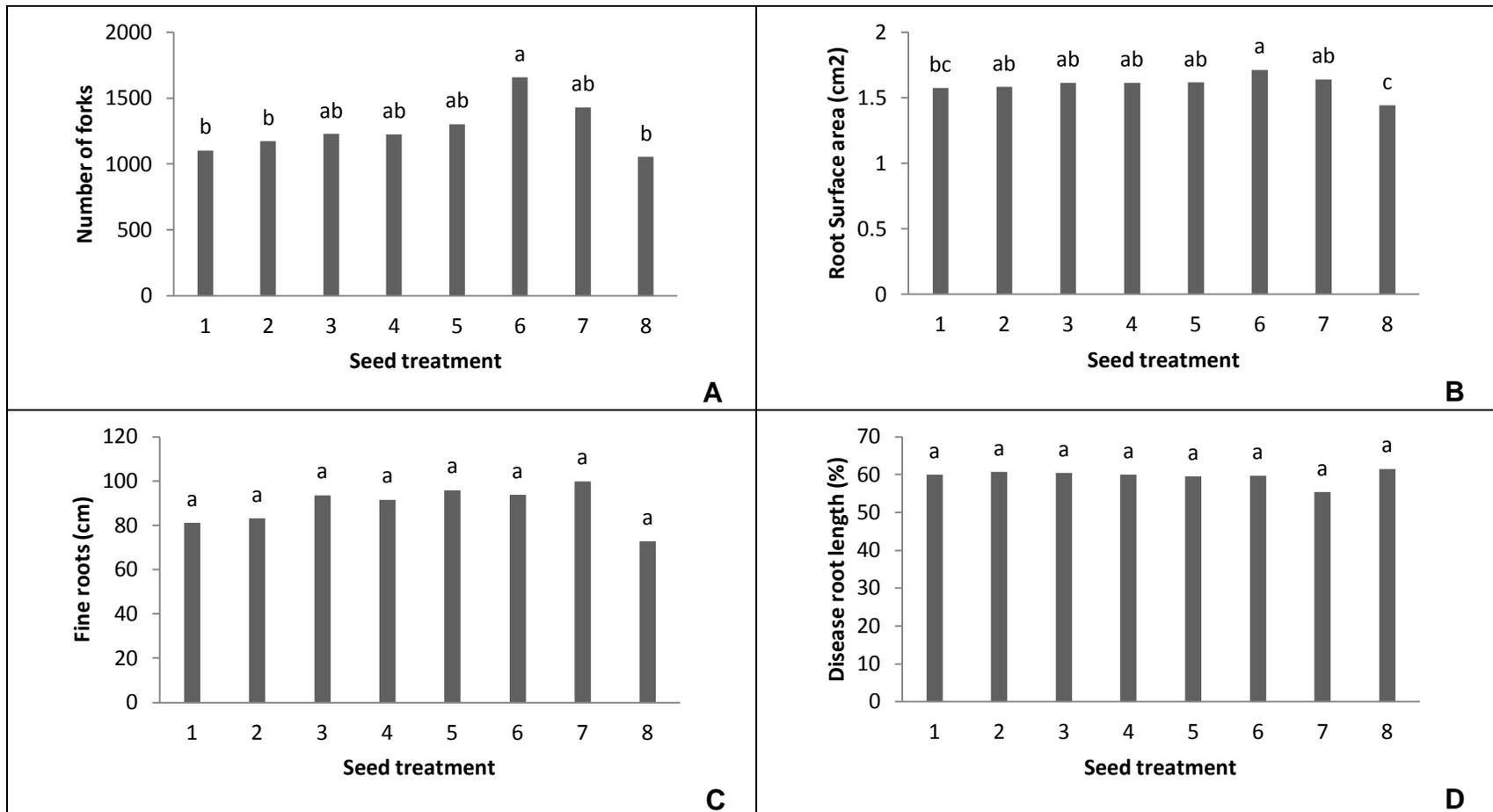


Figure 7. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium graminearum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

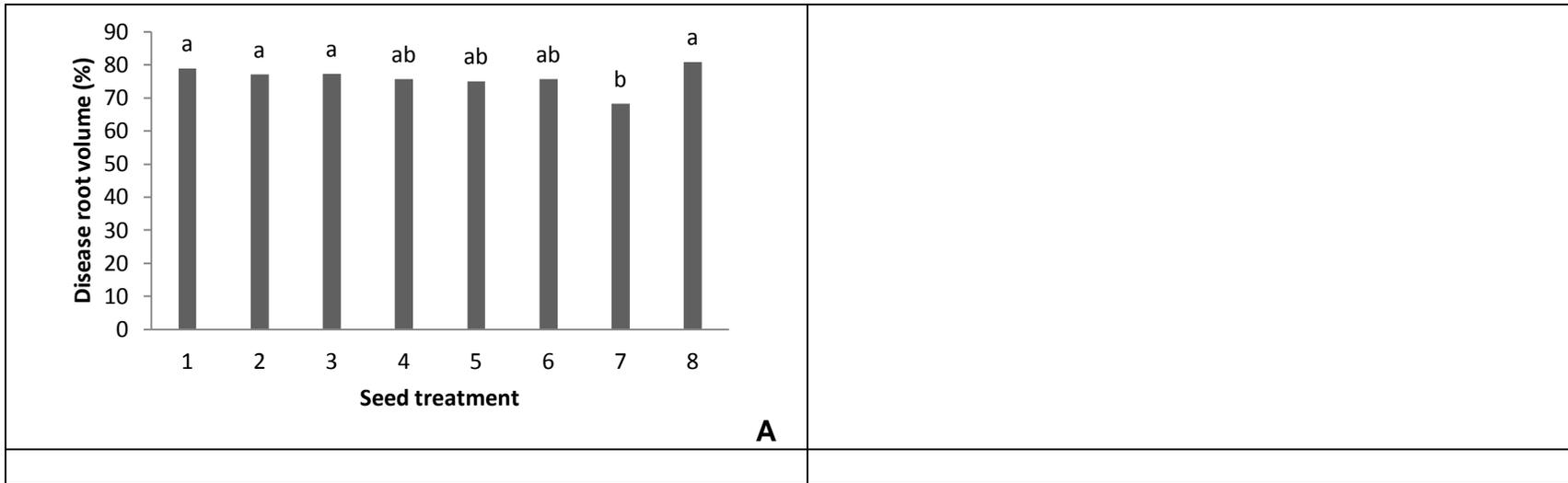


Figure 8. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium graminearum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

Table 5. *P*-values for effects of seed treatments and pathogen inoculation on seedling health variables for *Fusarium verticillioides* experiments. Analysis of variance was conducted with log transformed data except for disease root length (%) and disease root volume (%) which were not transformed.

Variables	Effects						
	Seed treatment (ST)	Fungus (F)	Nematode (N)	ST x F	ST x N	F x N	ST x F x N
Shoot length (cm)	ns	ns	ns	ns	ns	ns	ns
Shoot fresh (g)	ns	ns	ns	ns	ns	ns	ns
Shoot dry (g)	ns	ns	ns	ns	ns	ns	ns
Root fresh (g)	ns	ns	ns	ns	ns	ns	ns
Root dry (g)	ns	ns	ns	ns	ns	ns	ns
Root length (cm)	ns	<.0001	<.0001	0.001	ns	0.004	ns
Root volume (cm <sup>3</sup> )	ns	<.0001	0.001	0.01	ns	ns	ns
Tips	ns	<.0001	<.0001	0.004	ns	0.004	ns
Forks	ns	<.0001	<.0001	0.003	ns	0.005	ns
Surface area (cm <sup>2</sup> )	ns	<.0001	<.0001	0.001	ns	0.009	ns
Fine roots (cm)	ns	<.0001	<.0001	0.001	ns	0.001	ns
Disease root length (%)	ns	<.0001	0.04	ns	ns	0.009	ns
Disease root volume (%)	ns	<.0001	0.03	ns	ns	0.02	ns

\* ns = not significant ( $p>0.05$ )

Table 6. *P*-values for effects of seed treatments and pathogen inoculation on nematode extraction results for *Fusarium verticillioides* experiments.

	Effects		
	Seed treatment (ST)	Fungal inoculation (F)	ST x F
Nematodes from soil	0.0005	ns	ns
Nematodes from roots	0.003	ns	0.01

\* ns = not significant ( $p > 0.05$ )

Table 7. Number of *Pratylenchus penetrans* recovery from 100 cm<sup>3</sup> of soil and the roots therein and from 1 g of dried maize roots from treatments infested with the nematodes and with or without *Fusarium verticillioides* infestation

Treatment	Active ingredients	Nematodes / 100 cm <sup>3</sup> soil	Nematodes / g root
1	Fludioxonil + Mefenoxam + Azoxystrobin	47.7c	10abc
2	FMA <sup>a</sup> + Thiabendazole	49.7c	16ab
3	FMA + Thiamethoxam	77.7c	12ab
4	FMA + Thiamethoxam + Thiabendazole	42.2bc	18.7a
5	FMA + Abamectin	148.7a	0c
6	FMA + Abamectin + Thiabendazole	110.2a	0.2c
7	FMA + Abamectin + Thiamethoxam + Thiabendazole	140.2a	0c
8	Untreated	65.5bc	7.5c

<sup>a</sup> FMA = Maxim + Apron XL + Dynasty = Treatment 1

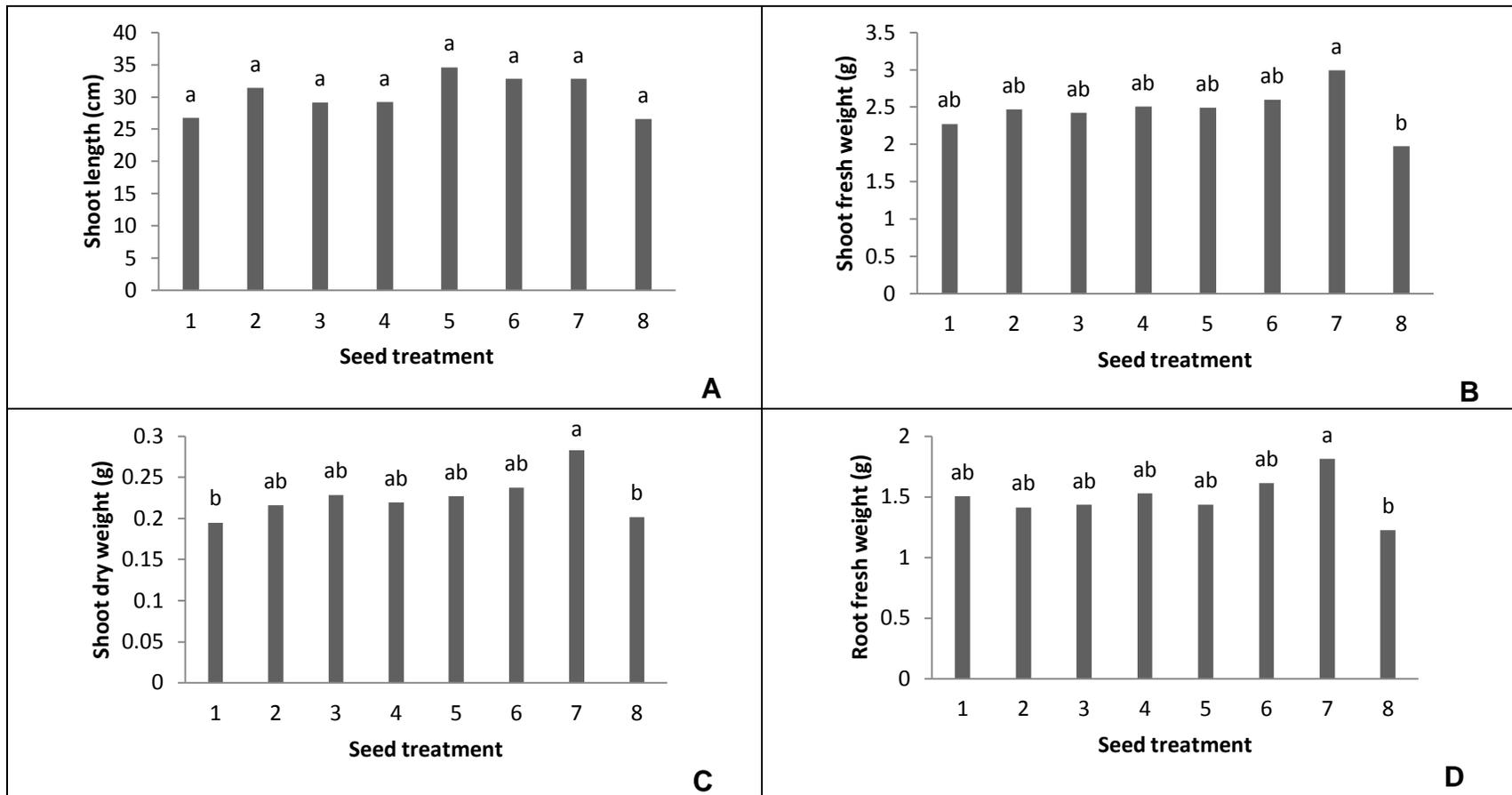


Figure 9. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Fusarium verticillioides* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

-In *F. graminearum* experiments, treatment 6 was usually best.

-In *F. verticillioides* experiments, treatment 7 was usually best.

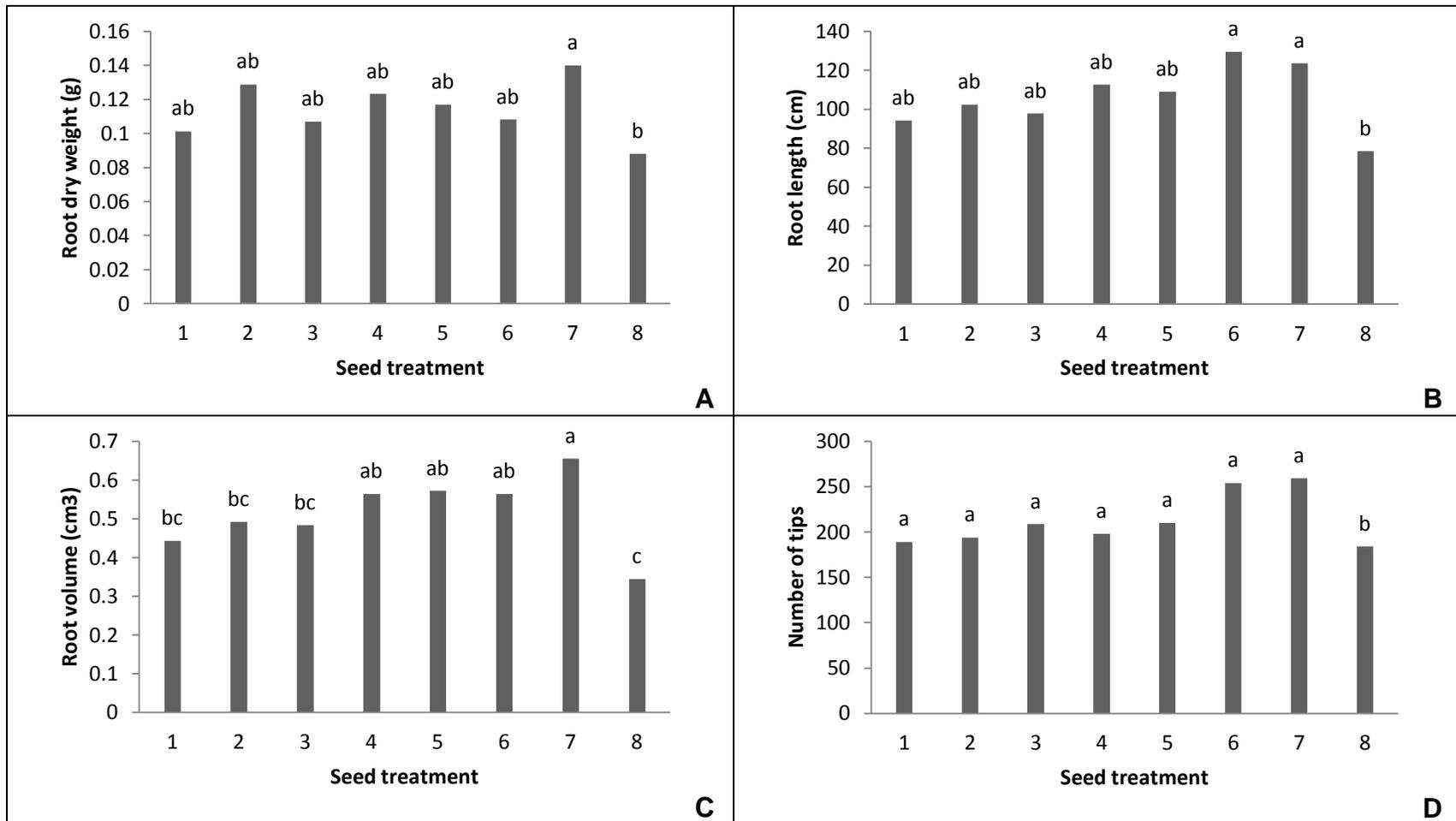


Figure 10. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Fusarium verticillioides* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

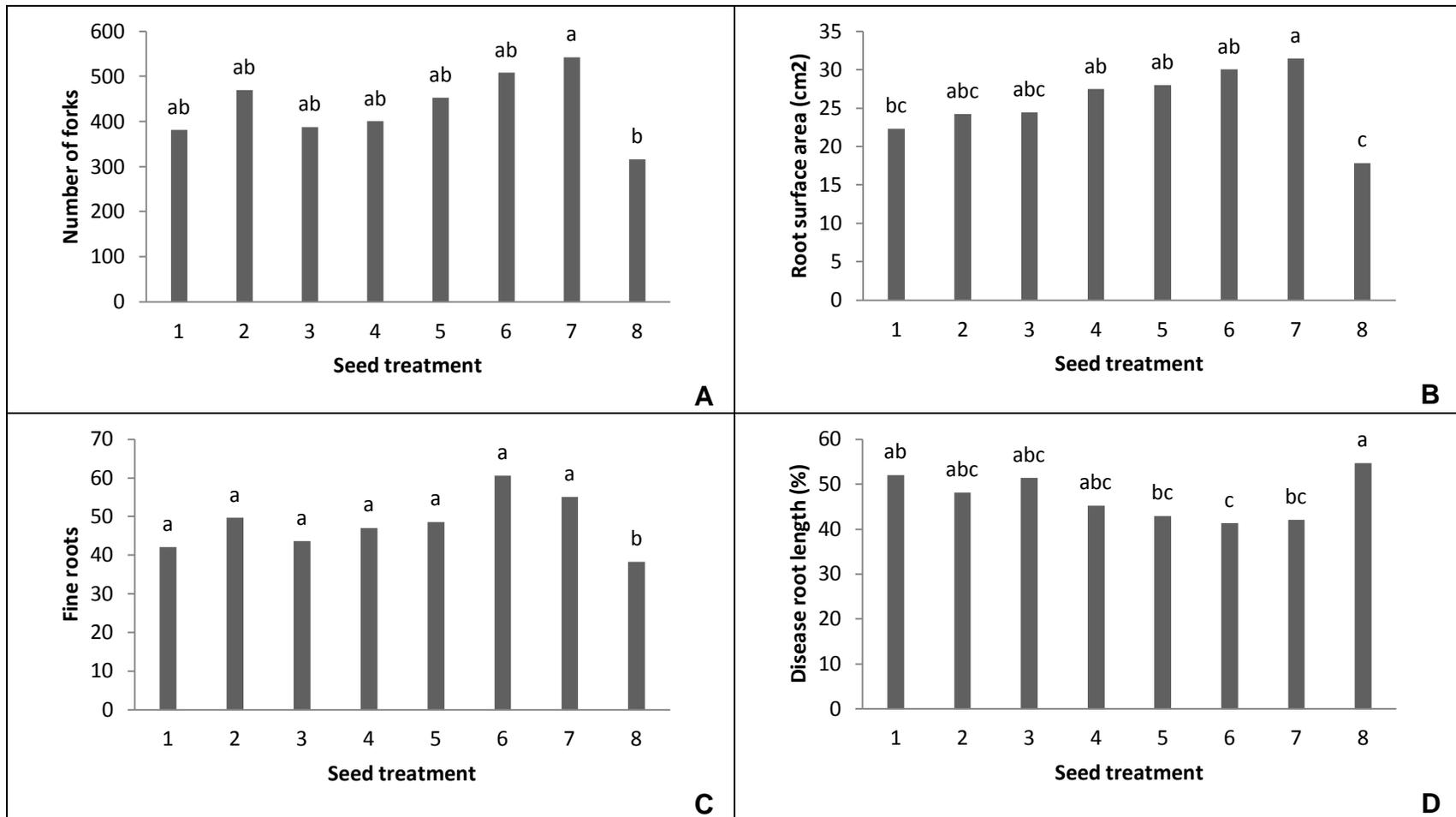


Figure 11. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Fusarium verticillioides* – infested sand.. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

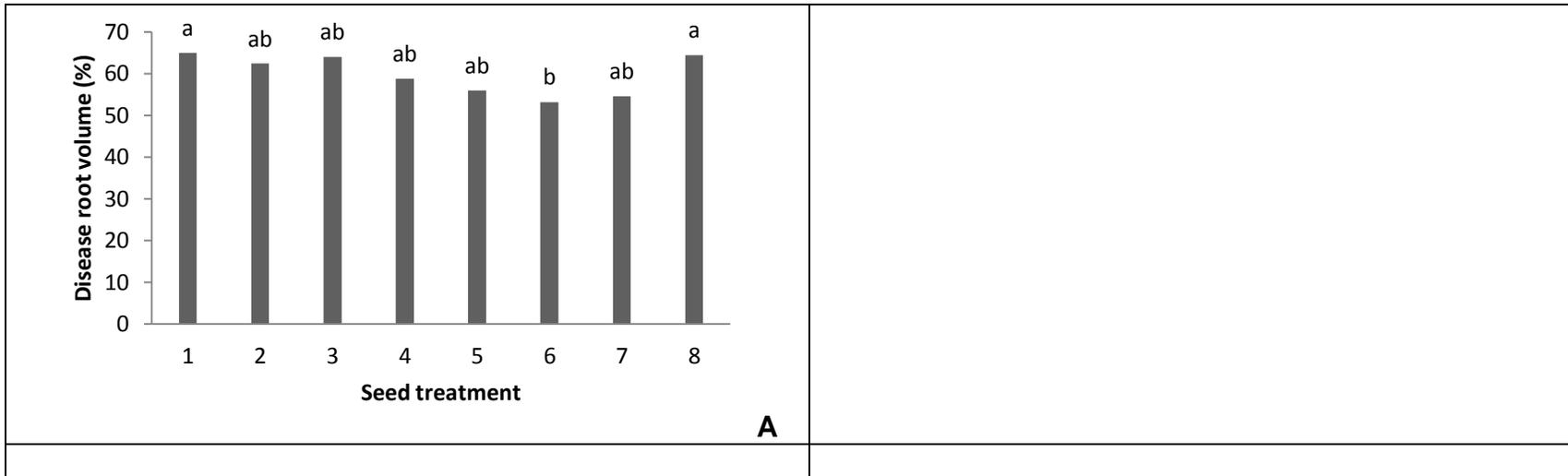


Figure 12. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Fusarium verticillioides* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

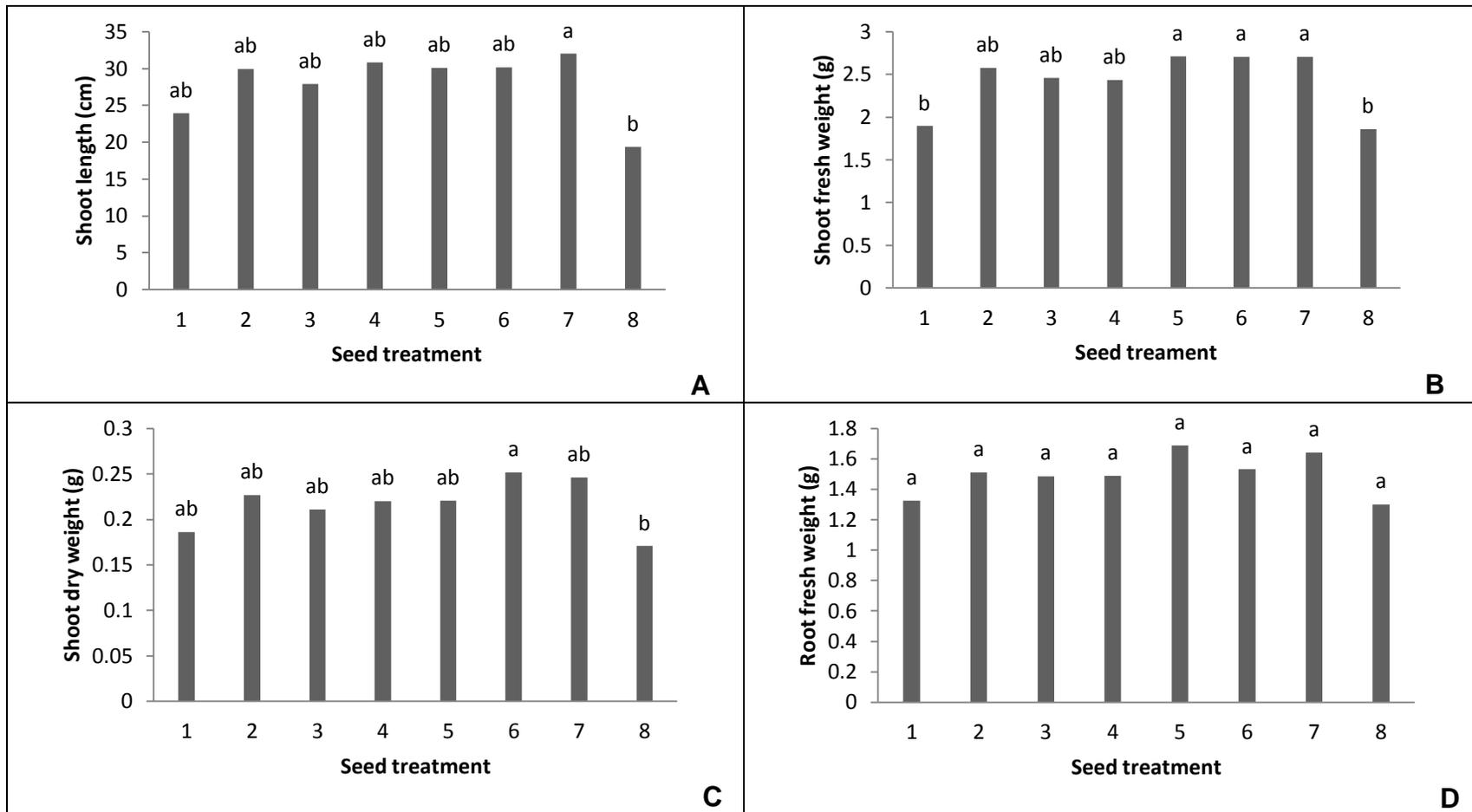


Figure 13. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium verticillioides*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

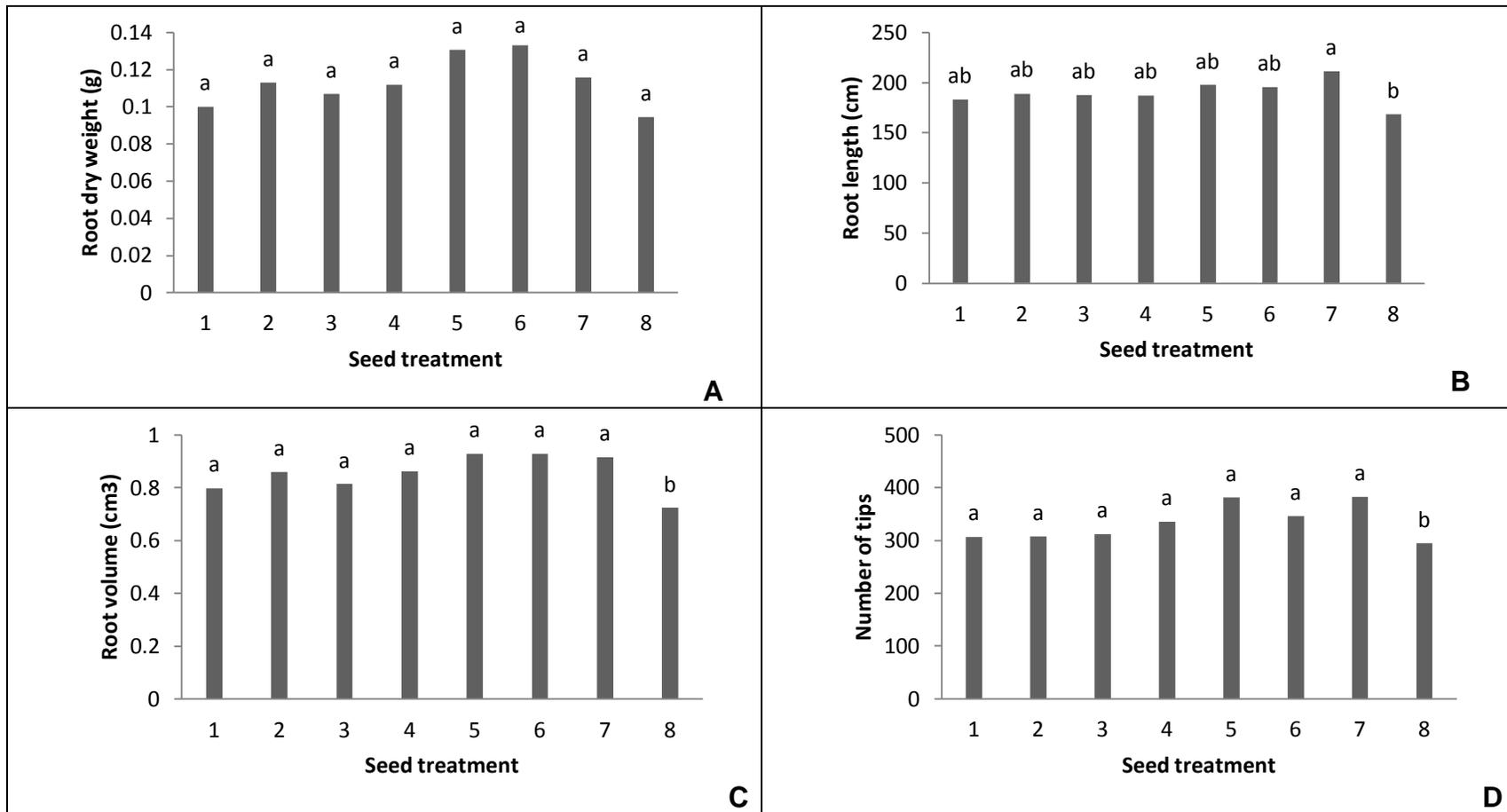


Figure 14. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium verticillioides*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

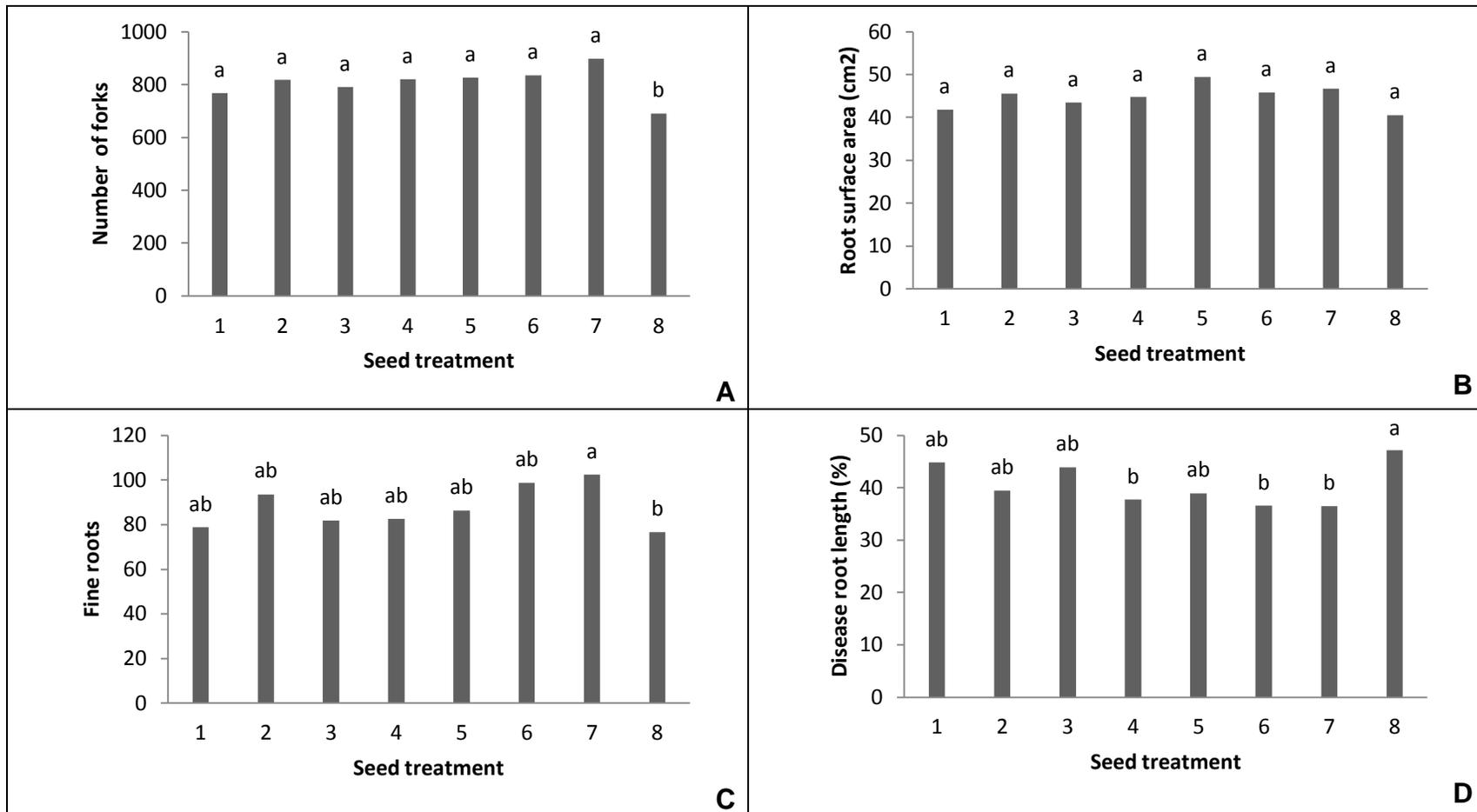
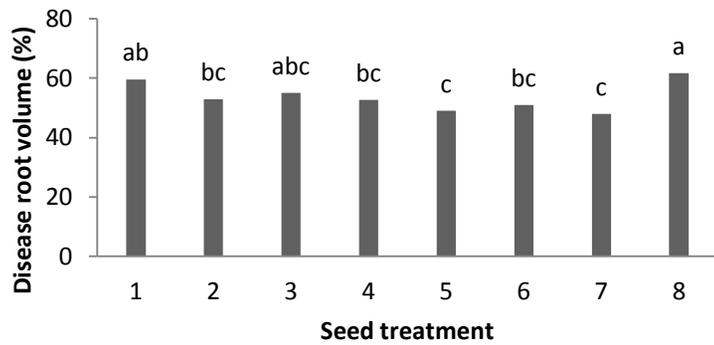


Figure 15. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium verticillioides*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .



**A**

Figure 16. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Fusarium verticillioides*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .



Figure 17. Pictures showing seed treatment control on maize seedlings. Treatment 5 = FMA + Abamectin (A), Treatment 6 = FMA + Abamectin + Thiabendazole (B), Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole (C), Treatment 8 = Untreated (D).

### CHAPTER 3.

#### ROOT-LESION NEMATODE INTERACTIONS WITH *PYTHIUM ULTIMUM* AND *RHIZOCTONIA SOLANI* ON MAIZE SEEDLINGS

M.P. da Silva and G. P. Munkvold. Department of Plant Pathology. Iowa State University.

Ames, IA 50011

Corresponding author: G.P. Munkvold. [munkvold@iastate.edu](mailto:munkvold@iastate.edu)

#### Abstract

Fungi and Oomycetes such as *Rhizoctonia solani* and *Pythium ultimum* play important roles in etiology of seedling diseases of maize. The roots of maize seedlings are typically attacked by a complex of pathogens and parasites, but interactions among these organisms are poorly understood. Several diseases on a variety of crops are caused by a fungus-nematode complex that often results in a synergistic interaction where in the crop loss is greater than expected from either pathogen alone or an additive effect of the two together. Root-rot pathogens such as *P. ultimum* and *R. solani* may be involved in nematode disease complexes. The objectives of this study were to measure interactions between *Pratylenchus penetrans* and fungal (*R. solani*) or Oomycete (*P. ultimum*) pathogens causing seedling disease symptoms on maize; assess the impact of nematode control with abamectin on these interactions; and evaluate added benefits of abamectin combined with fungicide seed treatment for seedling disease management. *Pythium ultimum* experiments were conducted twice in a growth chamber and *Rhizoctonia solani* experiments were conducted twice in the greenhouse at the Iowa State University, Ames, Iowa.

Experiments were conducted in 150 ml pots filled with an autoclaved sand-soil mixture combined with inoculum of *Pythium ultimum* or *Rhizoctonia solani* (colonized corn meal/sand mixture). A suspension of 4000 *P. penetrans* (adults and juveniles) was added to the pots at the time of planting. A factorial experimental design was used including 8 seed treatments x 4 pathogen combinations x 6 replicates. Four replicates of each treatment were harvested 30 days after planting. Shoot lengths, fresh and dry shoot and root weights, and visual root health scores were determined. Digital images of the root systems were recorded using a flatbed scanner and image analysis conducted with WinRhizo software (Regent Instruments Inc., Quebec, Canada); root length, volume, tips, branching, discoloration, surface area, and diameter class distribution were determined. The two remaining replicates were harvested 42 days after planting for extraction of nematodes from roots and soil. *Pratylenchus penetrans* had significant interactions with *P. ultimum* and this interaction caused more severe effects on plant growth and root structure than from *P. penetrans* or *P. ultimum* alone. Root health variables also were affected by *R. solani* and *P. penetrans* infestation in the greenhouse experiments, but no significant fungus-nematode interactions were observed. Analyses of roots by WinRhizo provided precise, quantitative estimates of changes in root structural characteristics and discoloration caused by the pathogens. Seed treatment combinations that included abamectin, or abamectin in combination with thiabendazole and thiamethoxam were the best treatments for improving most measures of root system and seedling health, and protecting the roots against nematode feeding.

### **Introduction**

Corn seed and seedlings are susceptible to infection by a number of soilborne pathogens, such as *Pythium*, *Fusarium*, *Rhizoctonia*, *Aspergillus*, *Penicillium*, and *Trichoderma*, that can cause seedling diseases. The symptoms are very similar among these pathogens, such as wilting ,

chlorosis/yellowing, root rot and poor root development, slow growth and stunting, and post-emergence damping-off. These symptoms can be more severe when soil temperatures are below 13° C, mainly because germination is greatly retarded in this temperature range. In addition, seedling diseases can reduce plant population at the level that replanting sometimes is necessary (Stack 2000; Vincelli 2008; Munkvold and Robertson 2009).

*P. ultimum* is widely distributed throughout the world and has a wide range of hosts, including important crops, such as corn and soybean. This pathogen can cause damping-off and root rot on plants, leading to tremendous economic loss (Hendrix and Campbell 1973). For example, *P. ultimum* is the common cause of a root rot of beans and peas in Washington and Wisconsin (Kraft and Burke 197; Adegbola and Hagedorn 1969; Kraft and Burke 1971).

Besides that, *Pythium* spp. are the most important soilborne pathogens associated with poor stands in maize and many other crops, especially in cold soils (Hoppe and Middleton 1950; Thomson, Athow et al. 1971). Most *Pythium* spp. can infect mainly juvenile or succulent tissues, and also commonly infect seed and radicals causing seed rot and pre-emergence damping-off (Hendrix and Campbell 1973). Thus, the damage caused by this pathogen can result in severe economic losses to maize and soybean growers in the north central region of the United States (Doupnik 1993). According to (Rao, Schmitthenner et al. 1978; Deep and Lipps 1996) several species of *Pythium* can cause disease on maize. For instance, based on (Rao, Schmitthenner et al. 1978; Lipps and Deep 1991) research in Ohio, they isolated and identified different species of *Pythium* from maize seedlings, such as *P. arrhenomanes* Drechs., *P. dissotocum* Drechs., *P. graminicola* Subramanian, *P. ultimum* Trow, and *P. torulosum*. Previous studies in Iowa have shown the existence of a *Pythium* complex on soybean, including *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. sylvaticum*, *P. ultimum* var. *sporangiferum*, and *P. ultimum* var.

*ultimum* (Rizvi and Yang 1996). Another study from (Rizvi, Yang et al. 1994), suggested that *P. irregulare* and *P. ultimum* could be the major species.

*Rhizoctonia solani* is the most widely recognized species of *Rhizoctonia*. It can cause damping-off of young seedlings, attacking below ground plant parts such as seeds, hypocotyls, and roots. *Rhizoctonia solani* can survive for several months in the soil in the form of sclerotia and also in colonized plant debris or on roots of weeds (Sumner 1996). Furthermore, the pathogen is adapted to a wide variety of environments and hosts, making control harder, even doing crop rotation may not be sufficient to reduce inoculum (Sumner and Bell 1986).

*R. solani* is divided into anastomosis groups (AG based on hyphal anastomosis and cultural characteristics (Ogoshi 1987; Sneh, Burpee et al. 1991; Dorrance, Kleinhenz et al. 2003). As a result, isolates within an AG may have analogous characteristics, such as the type of symptoms produced on a host and also host preferences (Anderson 1982; Sneh, Burpee et al. 1991).

Maize has been reported as a host plant of several *Rhizoctonia solani* anastomosis groups and subgroups. For example Summer and Bell (1982) reported AG 2-2 and AG-4 to be pathogenic on maize in Georgia. Similarly, Ithurrart, Buttner et al. (2004) clearly demonstrated that maize serves as a host plant for *Rhizoctonia solani* AG 2-2IIIB.

Fungi play an important role in disease etiology of several diseases caused by a fungus-nematode complex. The combination of nematode and fungus often results in a synergistic interaction where in the crop loss is greater than expected from either pathogen alone or an additive effect of the two together. Root-rot pathogens such as *Pythium ultimum* and *Rhizoctonia solani*, according to (Back, Haydock et al. 2002) can be involved in nematode disease complexes. For example, *Meloidogyne incognita* predisposes tomato and tobacco plants to

subsequent infection when exposed to either *R. solani* or *P. ultimum* (Nava 1970), although, when both of them are present, *R. solani* seems to be more aggressive than *P. ultimum* (Powell 1971). Also, based on results of Polychronopoulos, Houston et al. (1969), *Heterodera schachtii* (beet-cyst nematode) seems to facilitate the infection of sugar beet by *R. solani*.

*P. penetrans* is probably one of the most common species found on corn in the United States (Norton 1984) and on sweet corn in Eastern Canada (Potter and Townshend 1973). *Pratylenchus penetrans* is a destructive migratory endoparasite of root cortex. They can enter and migrate within roots and feed on various tissues, resulting in necrotic lesions on the root surface. As a result, infections can appear along the entire root length of the host plant, excluding root tips (Townshend and Stobbs 1981; Castillo, Vovlas et al. 1998); root lesions can fuse and become discolored with time, although the color of the lesion varies with the host (Townshend and Stobbs 1981).

Therefore, *P. penetrans* mechanism of pathogenesis is related to the way that the nematode can affect the host by either feeding on roots or as interact with other organisms in disease complexes such as involving fungi (Endo 1975). In fact, *P. penetrans* process of pathogenicity includes the predisposition of plants by nematode wounding roots. As a result of that, there is an increase susceptibility to a successive disease caused by others organism.

However, *P. penetrans* interactions with *R. solani* and *Pythium ultimum* have not been studied on maize and more research is needed in order to better understand the importance of these interactions. The objectives of this study were to measure interactions between *Pratylenchus penetrans* and fungal (*R. solani*) or Oomycete (*P. ultimum*) causing seedling disease symptoms on maize, especially root system effects; assess the impact of nematode

control with abamectin on these interactions; and evaluate added benefits of abamectin combined with fungicide seed treatment for seedling disease management.

### **Material and methods**

**General design:** *Pythium ultimum* experiments were conducted twice in a growth chamber and *Rhizoctonia solani* experiments were conducted twice in the greenhouse at the Iowa State University, Ames, Iowa. A full factorial experimental design was used. Experimental factors were seed treatment (eight treatments), nematode infestation (infested or not infested), and fungal infestation (infested or not infested). Seed of one maize hybrid (NK Brand hybrid N40T-GT, Syngenta Seeds, Golden Valley, MN) was treated with seven different combinations of active ingredients and a non-treated control was included (Table 1). Seed treatment rates used were the recommended rates for commercial use. Treatments were arranged in a growth chamber as a completely randomized block design with six replicates. Each pot (cone) was an experimental unit.

**Fungal and nematode infestation:** *Pythium ultimum* (isolate 350) (provided by Pioneer Hi-Bred, Int., Inc.) and *Rhizoctonia solani* (isolate 65L-2, AG 2-2 (Liu and Sinclair 1991) (provided by Drs. Wayne Pedersen and Carl Bradley, Univ. of Illinois), were used in the experiments. Inoculum of *P. ultimum* and *R. solani* isolates was prepared following the procedure described by Munkvold and O'Mara (2002), modified from that of (Desjardins, Plattner 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. Inoculum of *R. solani* was cultivated on potato dextrose agar (PDA) at 20°C in the dark. Mycelium from one 7-day-old culture (9-cm Petri dish) was cut into small pieces and mixed in each bag with the substrate mixture. Inoculum of *P. ultimum* was grown on potato dextrose agar (PDA) and incubated at

room temperature (22 to 25°C) for 7 days in the dark. After that, mycelium from one Petri dish was cut into small pieces and mixed in each bag mixture. The bags were then incubated in the dark at ambient temperature (20 to 24°C) for six days, with mixing every day. Autoclaved sand-soil (1 part soil: 2 parts sand) was mixed with *R. solani* or *P. ultimum* inoculum. The proportion was 30% of inoculum and 70% by volume of sand-soil mixture. Cones (150 ml) were filled with the mixture. A small piece of paper towel was placed in the bottom of each cone to partially retard drainage. One maize seed was placed in each cone. *Pratylenchus penetrans*, provided by Dr. A.E. MacGuidwin (University of Wisconsin, Madison), was cultured monoxenically (Layne and A.E. MacGuidwin 1994) on excised sweet corn roots in Gamborg's B-5 Medium with vitamins and without cytokinins or auxin (Gamborg, Murashige et al. 1976). Agar surfaces of 3-month-old cultures were rinsed with sterile distilled water to collect nematodes (Layne and A.E. MacGuidwin 1994). The nematode inoculum was prepared in water suspension (Martin, Riedel et al. 1982) in a total volume of 50 ml which was then diluted to achieve a density of 2000 nematodes/ml determined by nematode counting slide. Nematodes were added to the cones by injection of 2 ml suspension (4000 nematodes) using a microliter pipette at the time of planting (Saeed, MacGuidwin et al. 1999).

**Growing conditions:** *Pythium ultimum* experiments were conducted in a growth chamber under light supplied by cool white fluorescent and incandescent lamps with a photoperiod of 14 hours. Relative humidity was maintained at 99% and temperature was 22° C ( $\pm 0.1$  C). A temperature of 22°C throughout day and night was chosen to provide optimal maize growth and nematode reproduction. Relative humidity was adjusted to 70-80%. *Rhizoctonia solani* experiments were conducted in a greenhouse using artificial light for 14 h a day and temperature was 28°C ( $\pm 8$  C).

**Data collection and analysis:** Four replicates were harvested 30 days after planting. They were removed from the cones and the roots were well washed. Shoot lengths (flag leaf), fresh shoot and root weights were measured. Shoots and roots were oven-dried at 110°C for 24h and weighed.

To perform color and root morphology analyses for each treatment, roots were scanned and image analyses were conducted with the software WinRhizo 2008a (Regent Instruments Inc). The procedures were as follows: washed and intact roots were spread out in a transparent tray in order to avoid overlapping during scanning process. A blue piece of plastic served as image background. Image recording was performed at a resolution of 600 dpi using a 24bit color mode, and images were saved as TIFF (tagged image file format). All other scanner settings, such as dust removal, etc. were turned off. A Dell Precision T3500 was used to drive the scanner (an Epson Perfection V700 Photo – Dual Lens System). In order to perform color analyses with the WinRhizo software, the first step was calibrating the program to distinguish the background and the healthy and diseased roots. This was done by creating color classes and groups, the latter being a set of classes which defined the range of colors assigned to each class (healthy roots, diseased roots, or background). In addition to the color analyses, root morphology also was determined and the following measurements were recorded: total root length (cm), total surface area (cm<sup>2</sup>), total volume (cm<sup>3</sup>), number of tips, number of forks and length (cm) of fine roots (<0.5 mm diameter).

**Nematode extraction:** Two replicates per treatment were harvested after six weeks in order to extract nematodes from soil and roots. A 100 cm<sup>3</sup> 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). Roots were cut into small pieces (1 cm) long, mixed and

incubated in Baermann funnels for two days. After nematodes were collected, the roots were dried at 100°C for two days and weighed. The number of *P. penetrans* (juveniles and adults) was counted using an inverted microscope. The total number of nematodes present per pot was calculated based on soil volume and root weight. Also the number of *P. penetrans* per gram of dry root weight was determined.

**Statistical analysis:** Data collected were analysed by analysis of variance (ANOVA) for a completely randomized block design (SAS Inc., Cary, NC). Least significant differences ( $P \leq 0.05$ ) for comparing treatment means also were calculated according to the GLM procedure of SAS. In order to meet ANOVA assumptions regarding normality and equal variance, analyses of variance were performed on transformed ( $\log_{10}$ ) data except for disease root length (%), disease root volume (%) and nematode counts. Analysis of all main effects and interactions was conducted using all treatment combinations. Because of significant seed treatment by pathogen infestation interactions, seed treatment effects also were tested for inoculated treatments only, excluding the non-inoculated treatments. Seed treatment means were analyzed separately by inoculation treatment; fungal-inoculated plants alone and *P. penetrans*-inoculated plants alone.

## Results

### *Pythium ultimum* experiments

Infestation of the potting medium with *P. ultimum* significantly affected shoot dry weights ( $P=0.04$ ), numbers of root tips ( $P=0.05$ ), fine roots ( $P=0.03$ ), diseased root length ( $P=0.002$ ) and diseased root volume ( $P=0.009$ ) percentages. Nematode infestation only affected diseased root volume ( $P=0.03$ ). There were significant *P. ultimum* – *P. penetrans* interactions affecting shoot length ( $P=0.02$ ), shoot fresh weight ( $P=0.02$ ), shoot dry weight ( $P=0.02$ ), root fresh weight ( $P=0.02$ ), root length ( $P=0.02$ ), root volume ( $P=0.02$ ), number of tips ( $P=0.01$ ),

number of forks ( $P=0.01$ ), surface area ( $P=0.01$ ) and number of fine roots ( $P=0.04$ ) (Table 2). The effects on the remaining variables were not significant.

Analysis of variance for seed treatment effects on *P. ultimum*-infested plants indicated significant seed treatment effects for diseased root length and diseased root volume, but not for the other variables (Figures 1 to 4). Regarding to the nematode- inoculated plants, analysis of variance for seed treatment effects indicated significant seed treatment effects for shoot length, root length, root volume, number of tips, number of forks, root surface area, number of fine roots, disease root length and disease root volume. Treatments 6 (FMA + abamectin + thiabendazole) and 7 (FMA + abamectin + thiamethoxam + thiabendazole) usually resulted in the highest means for the various plant health variables.

There were significant effects of fungal infestation ( $P=0.04$ ) affecting the numbers of *Pratylenchus penetrans* extracted from soil (Table 3). The effects on the remaining variables were not significant (Tables 3 and 4).

### ***Rhizoctonia solani* experiments**

Fungal infestation had stronger effects than nematode infestation, affecting root length ( $P<0.0001$ ), root volume ( $P<0.0001$ ), number of tips ( $P<0.0001$ ), number of forks ( $P<0.0001$ ), surface area ( $P<0.0001$ ), number of fine roots ( $P<0.0001$ ), disease root length ( $P=0.01$ ) and disease root volume ( $P=0.006$ ). Nematode infestation significantly affected only shoot fresh weights ( $P=0.02$ ). Seed treatment significantly affected root length ( $P=0.006$ ), number of tips ( $P=0.01$ ), number of forks ( $P=0.004$ ), surface area ( $P=0.008$ ) and fine roots ( $P=0.01$ ). Seed treatment interacted with nematode infestation affecting root dry weight ( $P=0.05$ ), numbers of tips ( $P=0.04$ ), and the number of forks ( $P=0.04$ ). The effects on the remaining variables were not significant (Table 5).

Analysis of variance for seed treatment effects on fungus-infested plants indicated significant seed treatment effects for root length, root volume, number of tips, number of forks, root surface area, number of fine roots, disease root length and disease root volume, but not for the other variables (Figures 10 B,C,D, 11 and 12). Regarding the nematode-infested plants, analysis of variance for seed treatment effects indicated significant effects for shoot fresh weight, shoot dry weight, root fresh and dry weights, root length, root volume, number of tips, number of forks, root surface area, number of fine roots and disease root volume (Figures 13 B,C,D, 14, 15 A,B,C and 16). Treatments 6 (FMA + Abamectin + Thiabendazole) and 7 (FMA + Abamectin + Thiamethoxam + Thiabendazole) usually resulted in the highest means for the various plant health variables followed by treatments 2 (MAD + Thiabendazole) and 4 (MAD + Thiamethoxam + Thiabendazole).

There were significant effects of seed treatment ( $P=0.002$ ), fungal inoculation ( $P=0.002$ ) and also a significant interaction between seed treatment and fungal inoculation ( $P=0.0006$ ) affecting the numbers of nematodes extracted from soil. Furthermore, there was a significant effect of fungal inoculation ( $P=0.03$ ) affecting the numbers of nematodes extracted from the roots (Table 6). The only seed treatment effect on nematode recovery was higher recovery from soil for treatment 6 vs. the untreated control (Table 7).

### Discussion

Infestation of the potting medium with *P. ultimum*, *R. solani*, or *P. penetrans* had significant detrimental effects on seedling and root health variables measured in this study. These effects were less dramatic for *P. ultimum* than for *R. solani*, but *P. ultimum* displayed significant interactions with *P. penetrans*, whereas *R. solani* did not. The results demonstrate that *P. penetrans* can interact with *P. ultimum* on maize and this interaction can cause more severe

effects on plant growth and root structure than from nematodes or fungi inoculation alone (Table 2). This is consistent with Palmer and MacDonald (1974), who suggested that the reduction in fresh weights of roots, shoots and dry weights of maize seedlings can be attributed to the nematode interaction with *P. ultimum* inoculation.

Noninoculated treatments were not affected by seed treatment according to the variables used in this study. There were no negative effects concerning phytotoxicity, such as lower germination and/or stunting plants.

Analyses of roots by WinRhizo software (Regent Instruments Inc., Quebec, Canada), provided precise quantitative estimates of root damage caused by *P. ultimum* for diseased root length and diseased root volume (Figures 3 D and 4 A). These results were consistent with Higginbotham, Paulitz et al. (2004), that reported that *Pythium ultimum* caused a significant decrease in the number of root tips on wheat cultivars.

The number of *P. penetrans* recovered from soil and from the maize roots was lower than expected and there were few differences among treatments. A previous investigation by Rotenberg, MacGuidwin et al. (2004) also recovered low numbers of nematodes from roots. *Pythium ultimum* and *R. solani* inoculum made with corn meal may have a detrimental effect on the ability of the nematodes to infect and reproduce (G.L. Tylka, pers. comm.). Nevertheless, seed treatments combinations including abamectin (5, 6 and 7) significantly improved root health characteristics compared to the untreated control in *P. penetrans*-infested treatments (Figures 5 to 8 and 13 to 16).

Abamectin is a broad-spectrum nematicide providing protection against a wide range of parasitic nematodes, including: lesion, root-knot, stubbly-root, lance, sting, dagger, needle, ring, spiral and stunt nematodes. However, abamectin used as a seed treatment nematicide provide

early season, not season-long, nematode protection and the duration of protection is not specified or known.

Although seed treatment effects did not present dramatic results regarding some variables for fungus-inoculated and nematode-inoculated plants, this study showed that there is a benefit of abamectin in combination with fungicide seed treatment in order to improve root health characteristics in the presence of both the nematode and *R. solani* or *P. ultimum*. In fact, those combinations have shown efficacy reducing disease root length and also disease root volume (Figures 3 D, 4 A, 7 D and 8 A).

Seed treatment effects in the *P. ultimum* experiments were moderate, and this may be due to relatively low aggressiveness of *P. ultimum* in our experiments. It is likely that the low aggressiveness of the *Pythium ultimum* inoculum was due to the temperature used in the growth chamber. According to several researchers (Hooker 1953; Thomson, Athow et al. 1971; Foley 1980; Crawford 1982) *Pythium* spp. usually cause seedling disease (pre- and postemergence damping-off) under a low temperature environment (10 to 15°C). Low temperature may affect physiological processes related to the seed germination and this can be unfavorable for seedling growth. As a consequence, these factors together may predispose younger seedlings and germination seeds more susceptible to *Pythium* spp. (Zhang and Yang 2000). However, according to Acosta and Malek (1979), the optimum temperature for *P. penetrans* range among 22° to 25°C. Therefore, the interaction between the two organisms is probably affected by the temperature; it may have been too warm for significant seedling disease caused by *Pythium ultimum*. The results suggest that lesion nematodes could be important for enhancing *P. ultimum* damage when temperature conditions are not optimal for *P. ultimum* to cause seedling disease on its own. Temperature can be critical in some nematode-fungus interactions (France and Abawi

1994; Walker, Kirkpatrick et al. 2000). In order to fully understand lesion nematode interactions with seedling pathogens, these studies should be repeated under a range of temperatures.

Several reports have been published related to the interaction between a plant-parasitic nematode and *R. solani*. For example, (LaMondia and Martin 1989) reported the black root-rot of strawberries, caused by *Rhizoctonia* spp, was more severed in the presence of *Pratylenchus penetrans*. However, few studies relate to interactions between nematodes and fungi on maize seedlings. In fact, as far as we know this study is the first one investigating interactions between *P. penetrans* and *R. solani* on maize. Although no significant fungus-nematode interactions were observed, seed treatment combination 7 (FMA + abamectin + thiabendazole + thiamethoxam) was the most consistent treatment for improving root health variables compared to the untreated control in *R. solani*-infested treatments (Figures 11 D and 12).

The results from root health parameters also clearly demonstrate the aggressiveness of the *Rhizoctonia solani* related to the diseased root length and diseased root volume. Recently Schroeder and Paulitz (2008) found that WinRhizo provided accurate estimates of root damage caused by *Rhizoctonia* spp. in wheat and barley. Paulitz, Smith et al. (2003), working with *Rhizoctonia oryzae* on wheat and barley cultivars, reported that root scanning technology of WinRhizo is an improvement over root dry weights.

Overall seed treatment effects were not common when non-infested treatments were included in the analysis. Seed treatment significantly affected some variables in *R. solani* experiments but not in *P. ultimum* experiments. *R. solani* results demonstrated a significant impact concerning seed treatment interactions with nematode inoculum.

Highly variable greenhouse temperatures also may have affected nematode activity and results of the nematode-infested treatments. To our knowledge, this study is the first one working on the *P. penetrans* interactions between *P. ultimum* and *R. solani* on maize seedlings under controlled conditions. Results suggest that the importance of interactions between *P. penetrans* and maize seedling pathogens varies among pathogens. Interactions between *P. penetrans* and *P. ultimum* indicate that nematode feeding can be an important factor in enhancing damage by this Oomycete pathogen at temperatures above the optimum for *P. ultimum* alone. This could have important implications for seedling disease in later-planted maize crops, or in those planted in warmer areas. Additionally, *P. ultimum* is a pathogen on the roots of older maize plants, and this activity may be enhanced by *P. penetrans*.

#### **Literature cited**

- Acosta, N. and R. B. Malek (1979). Influence of temperature on population development of eight species of *Pratylenchus* on soybean. *Nematology* 11: 229-232.
- Adegbola, M. O. K. and D. J. Hagedorn (1969). Symptomatology and epidemiology of *Pythium* bean blight. *Phytopathology* 59: 1113-1118.
- Anderson, N. A. (1982). The genetics and pathogenicity of *Rhizoctonia solani*. *Annu. Rev. Phytopathol* 20: 329-347.
- Back, M. A., P. P. J. Haydock, et al. (2002). Disease complexes involving plant parasitic nematodes and soilborne pathogens. *Plant Pathology* 51: 683-697.
- Broders, K. D., P. E. Lipps, et al. (2007). Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in ohio. *Plant Disease* 91: 727-735.
- Chand, T., C. Logan, et al. (1985). Modes of penetration of *Rhizoctonia solani* in potato sprouts. *Annals of Biology* 1: 1-6.

- Crawford, R. P. (1982). Comparison of *Pythium ultimum* resistance and cold tolerance in maize. M.S. thesis. Iowa State University, Ames.
- Deep, I. W. and P. E. Lipps (1996). Recovery of *Pythium arrhenomanes* and its virulence to corn. *Crop Prot* 15: 85-90.
- Desjardins, A. E., R. D. Plattner, et al. (1995). Genetic analysis of fumonisin production and virulence of *Giberella fujikuroi*, mating population A (*Fusarium moniliforme*) on maize (*Zea mays*) seedlings. *Applied. Environ. Microbiol* 61: 79-86.
- Dorrance, A. E., M. D. Kleinhenz, et al. (2003). Temperature, moisture, and seed treatment effects on *Rhizoctonia solani* root rot of soybean. *Plant Disease* 87: 533-538.
- Doupnik, B. J. (1993). Soybean production and disease loss estimates for North Central United States from 1989 to 1991. *Plant Disease* 77: 1170-1171.
- Foley, D. C. (1980). Resistance to *Pythium debaryanum* in *Zea mays* seedlings. *Proc. Ia. Acad. Sci.* 87: 134-138.
- France, R. A. and G. S. Abawi (1994). Interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *phaseoli* on selected bean genotypes. *Nematology* 26: 467-474.
- Gamborg, O. L., T. Murashige, et al. (1976). Plant tissue culture media. *In Vitro* 12: 473-8.
- Hendrix, F. F. and W. A. Campbell (1973). *Pythium* as plant pathogens. *Annu. Rev. Phytopathol* 11: 77-98.
- Higginbotham, R. W., T. C. Paulitz, et al. (2004). Evaluation of adapted wheat cultivars for tolerance to *Pythium* root rot. *Plant Disease* 88: 1027-1032.
- Hooker, A. L. (1953). Relative pathogenicity of *Pythium* species attacking seedling corn. *Proc. Ia. Acad. Sci.* 60: 163-166.

- Hoppe, P. E. and J. T. Middleton (1950). Pathogenicity and occurrence in Wisconsin soils of *Pythium* species which cause seedling disease in corn. *Phytopathology* (Abstr) 40: 13.
- Ithurrart, M. E. F., G. Buttner, et al. (2004). *Rhizoctonia* root rot in sugar beet (*Beta vulgaris* ssp. *altissima*) - Epidemiological aspects in relation to maize (*Zea mays*) as a host plant. *Journal of Plant Disease and Protection* 111: 302-312.
- Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for extracting nematodes from soil. *Plant Disease Reporter* 48: 692.
- Kraft, J. M. and D. W. Burke (197). *Pythium ultimum* as a root pathogen of beans and peas in Washington. *Plant Disease* 55: 1056-1060.
- Kraft, J. M. and D. W. Burke (1971). *Pythium ultimum* as a root pathogen of beans and peas in Washington. *Plant Disease* 55: 1056-1060.
- LaMondia, J. A. and S. B. Martin (1989). The influence of *Pratylenchus penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. *Plant Disease* 73: 107-110.
- Layne, T. L. and A.E. MacGuidwin (1994). Recovery of *Pratylenchus penetrans* from plant tissue cultures. *Nematology*: 26-557 (Abstr.).
- Lipps, P. E. and I. W. Deep (1991). Influence of tillage and crop rotation on yield, stalk rot, and recovery of *Fusarium* and *Trichoderma* spp. from corn. *Plant Disease* 75: 828-833.
- Liu, Z. and J. B. Sinclair (1991). Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. *Plant Disease* 75: 682-687.
- Martin, M. J., R. M. Riedel, et al. (1982). *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. *Phytopathology* 72: 640-644.

- Munkvold, G. P. and A. Robertson (2009). Check general root and mesocotyl health when assessing corn stands. Integrated Crop management - Iowa State University - University Extension: <http://www.extension.iastate.edu/CropNews/2009/0519robertson.htm>.
- Nava, C. (1970). Influence of *Meloidogyne incognita* on root rot development by *Rhizoctonia solani* and *Pythium ultimum* in tomato. M.S. thesis. N. C. State Univ., Raleigh: 41.
- Ogoshi, A. (1987). Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annu. Rev. Phytopathol 25: 125-143.
- Palmer, L. T. and D. H. MacDonald (1974). Interactions of *Fusarium* spp. and certain plant parasitic nematodes on maize. Phytopathology 64: 14-17.
- Paulitz, T. C., J. D. Smith, et al. (2003). Virulence of *Rhizoctonia oryzae* on wheat and Barley cultivars from the pacific northwest. Plant Disease 87: 51-55.
- Polychronopoulos, A. B., B. R. Houston, et al. (1969). Penetration and development of *Rhizoctonia solani* in sugar beet seedlings infected with *Heterodera schachtii*. Phytopathology 59: 482-485.
- Powell, N. T. (1971). Interactions between nematodes and fungi in disease complexes. Annu. Rev. Phytopathol 9: 253-274.
- Ramsey, G. B. (1917). A form of potato disease caused by *Rhizoctonia solani*. Journal of Agricultural Research 9: 421-426.
- Rao, B., A. F. Schmitthenner, et al. (1978). Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. Phytopathology 68: 1557-1563.
- Rizvi, S. S. A. and X. B. Yang (1996). Fungi associated with soybean seedling disease in Iowa. Plant Disease 80: 57-60.

- Rizvi, S. S. A., X. B. Yang, et al. (1994). Occurrence of *Pythium* species isolate diseased soybean seedlings in Iowa soybean fields. *Phytopathology* (Abstr) 84: 1123.
- Rotenberg, D., A. E. MacGuidwin, et al. (2004). Interaction of spatially separated *Pratylenchus penetrans* and *Verticillium dahliae* on potato measured by impaired photosynthesis. *Plant Pathology* 53: 294-302.
- Saeed, I. A. M., A. E. MacGuidwin, et al. (1999). Limitation to photosynthesis in *Pratylenchus penetrans* - and *Verticillium dahliae* - infected potato. *Crop Science* 39: 1340-1346.
- Schroeder, K. L. and T. C. Paulitz (2008). Effect of inoculum density and soil tillage on the development and severity of *Rhizoctonia* root rot. *Phytopathology* 98: 304-314.
- Sneh, B., L. Burpee, et al. (1991). Identification of *Rhizoctonia* species. American Phytopathology Society.
- Stack, J. (2000). Management of seed and seedling diseases of cor. Nebraska Cooperative Extension NF00-427.
- Summer, D. R. and D. K. Bell (1982). Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zea*. *Phytopathology* 72: 86-91.
- Sumner, D. R. (1996). Sclerotia formation by *Rhizoctonia* species and their survival. - In: *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Second International Symposium on *Rhizoctonia*: 207-216.
- Sumner, D. R. and D. K. Bell (1986). Influence of crop rotation on severity of corn and brace root rot caused in corn by *Rhizoctonia solani*. *Phytopathology* 76: 248-252.
- Thomson, T. B., K. L. Athow, et al. (1971). The effect of temperature on the pathogenicity of *Pythium aphanidermatum*, *P. debaryanum*, and *P. ultimum* on soybean. *Phytopathology* 61: 933-935.

- Vincelli, P. (2008). Seed and seedling diseases of corn. UK Cooperative extension service - University of Kentucky - College of Agriculture.
- Walker, N. R., T. L. Kirkpatrick, et al. (2000). Influence of *Meloydogyne incognita* and *Thielaviopsis basicola* populations on early-season disease development and cotton growth. *Plant Disease* 84: 449-453.
- Zhang, B. Q. and X. B. Yang (2000). Pathogenicity of *Pythium* populations from corn-soybean rotations fields. *Plant Disease* 84: 94-99.

Table 1. Seed treatments used in the experiments. Treatment 1 is the commercial standard and Treatments 2 through 7 include fludioxonil, mefenoxam, and azoxystrobin (FMA) at the same rates as Treatment 1.

Treatment	Active ingredients	Chemical group	Formulation (%)	Brand name	Rate
1	Fludioxonil	Phenylpyrrole	40.3	Maxim	2.5g/100kg
	Mefenoxam	Phenylamide	1.1	Apron XL	2g/100kg
	Azoxystrobin	Strobilurin	9.6	Dynasty	1g/100kg
2	FMA <sup>a</sup> + Thiabendazole	Benzimidazole	42.3		20g/100kg
3	FMA + Thiamethoxam	Neonicotinoid	47.6	Cruiser	0.25mg/seed
4	FMA + Thiamethoxam + Thiabendazole	Neonicotinoid Benzimidazole	47.6 42.3	Cruiser	0.25mg/seed
	FMA + Abamectin	Avermectin	46.3	Avicta	0.25mg/seed
6	FMA + Abamectin + Thiabendazole	Avermectin Benzimidazole	12.4	Avicta	0.25mg/seed
	FMA + Abamectin + Thiamethoxam + Thiabendazole	Avermectin Neonicotinoid Benzimidazole	12.4	Avicta Cruiser	0.25mg/seed
8	Untreated		0		

<sup>a</sup>FMA = fludioxonil + mefenoxam + azoxystrobin = Treatment 1

Table 2. *P*-values for effects of seed treatments and pathogen inoculation on seedling health variables for *Pythium ultimum* – inoculated plants. Analysis of variance was conducted with log transformed data except for diseased root length (%) and diseased volume (%) which were not transformed.

Variables	Effects						
	Seed treatment (ST)	Pythium (P)	Nematode (N)	ST x P	ST x N	F x N	ST x P x N
Shoot length (cm)	ns	ns	ns	ns	ns	0.02	ns
Shoot fresh (g)	ns	ns	ns	ns	ns	0.02	ns
Shoot dry (g)	ns	0.04	ns	ns	ns	0.02	ns
Root fresh (g)	ns	ns	ns	ns	ns	0.02	ns
Root dry (g)	ns	ns	ns	ns	ns	ns	ns
Root length (cm)	ns	ns	ns	ns	ns	0.02	ns
Root volume (cm <sup>3</sup> )	ns	ns	ns	ns	ns	0.02	ns
Tips	ns	0.05	ns	ns	ns	0.01	ns
Forks	ns	ns	ns	ns	ns	0.01	ns
Surface area (cm <sup>2</sup> )	ns	ns	ns	ns	ns	0.01	ns
Fine roots (cm)	ns	0.03	ns	ns	ns	0.04	ns
Disease root length (%)	ns	0.002	ns	ns	ns	ns	ns
Disease root volume (%)	ns	0.009	0.03	ns	ns	ns	ns

\* ns = not significant ( $p > 0.05$ )

Table 3. *P*-values for effects of seed treatments and pathogen inoculation on nematode extraction results for *Pythium ultimum* experiments.

	Effects		
	Seed treatment (ST)	Pythium (P)	ST x P
Nematodes from soil	ns	0.04	ns
Nematodes from roots	ns	ns	ns

\* ns = not significant ( $p > 0.05$ )

Table 4. Number of *Pratylenchus penetrans* recovery from 100 cm<sup>3</sup> of soil and the roots therein and from 1 g of dried maize roots infested with the nematodes and / or *Pythium ultimum*.

Treatment	Active ingredients	Nematodes / 100 cm <sup>3</sup> soil	Nematodes / g root
1	Fludioxonil + Mefenoxam + Azoxystrobin	13.0ab	0.8a
2	FMA <sup>a</sup> + Thiabendazole	16.7ab	0.7a
3	FMA + Thiamethoxam	6.5b	0.1a
4	FMA + Thiamethoxam + Thiabendazole	15.2ab	0.2a
5	FMA + Abamectin	21.0a	0.0a
6	FMA + Abamectin + Thiabendazole	20.3a	0.0a
7	FMA + Abamectin + Thiamethoxam + Thiabendazole	14.7ab	0.0a
8	Untreated	10.2ab	0.0a

<sup>a</sup> FMA = fludioxonil + mefenoxam + azoxystrobin = Treatment 1

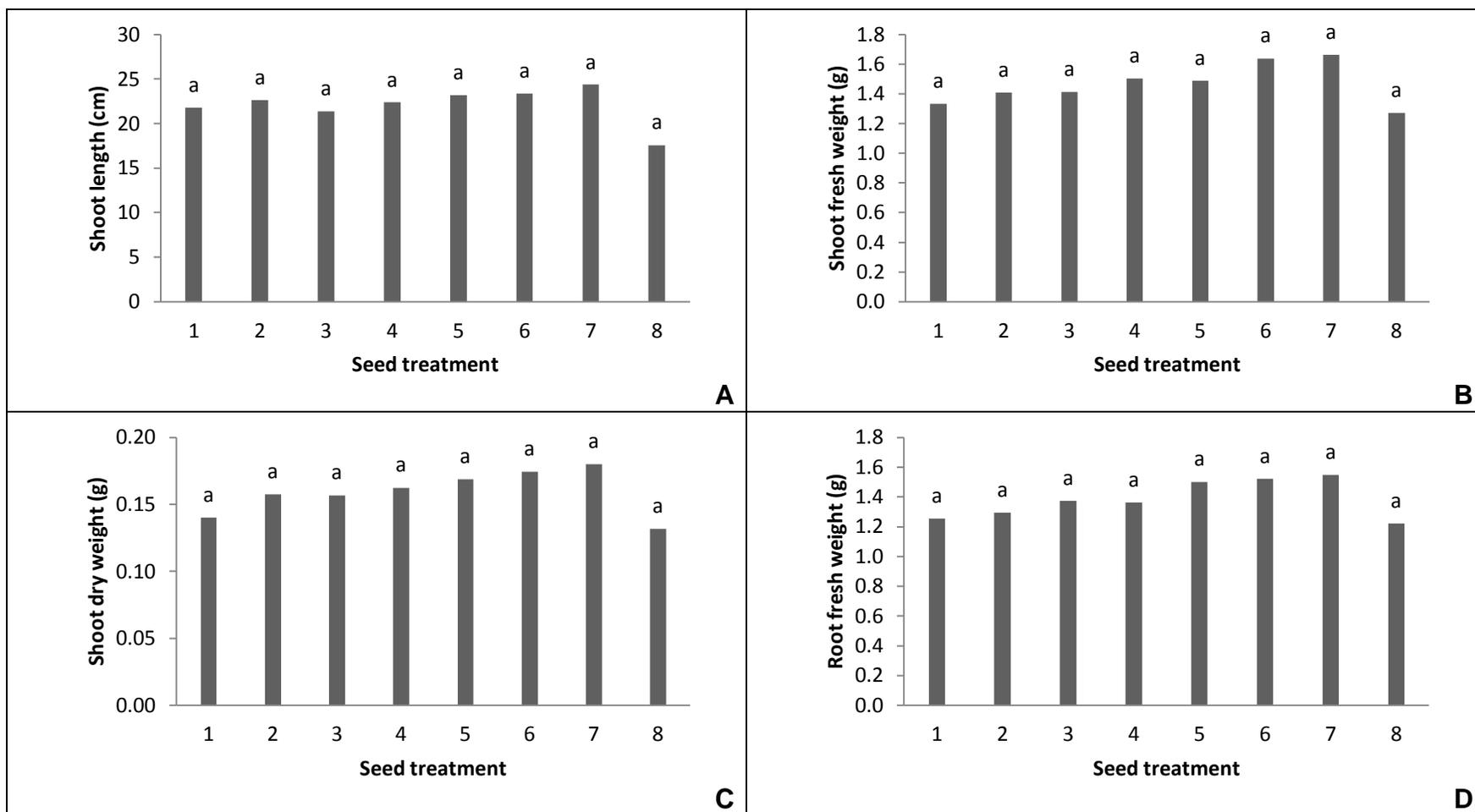


Figure 1. Shoot length means (A), shoot fresh weight means (B), shoot dry weight means (C), and root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Pythium ultimum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

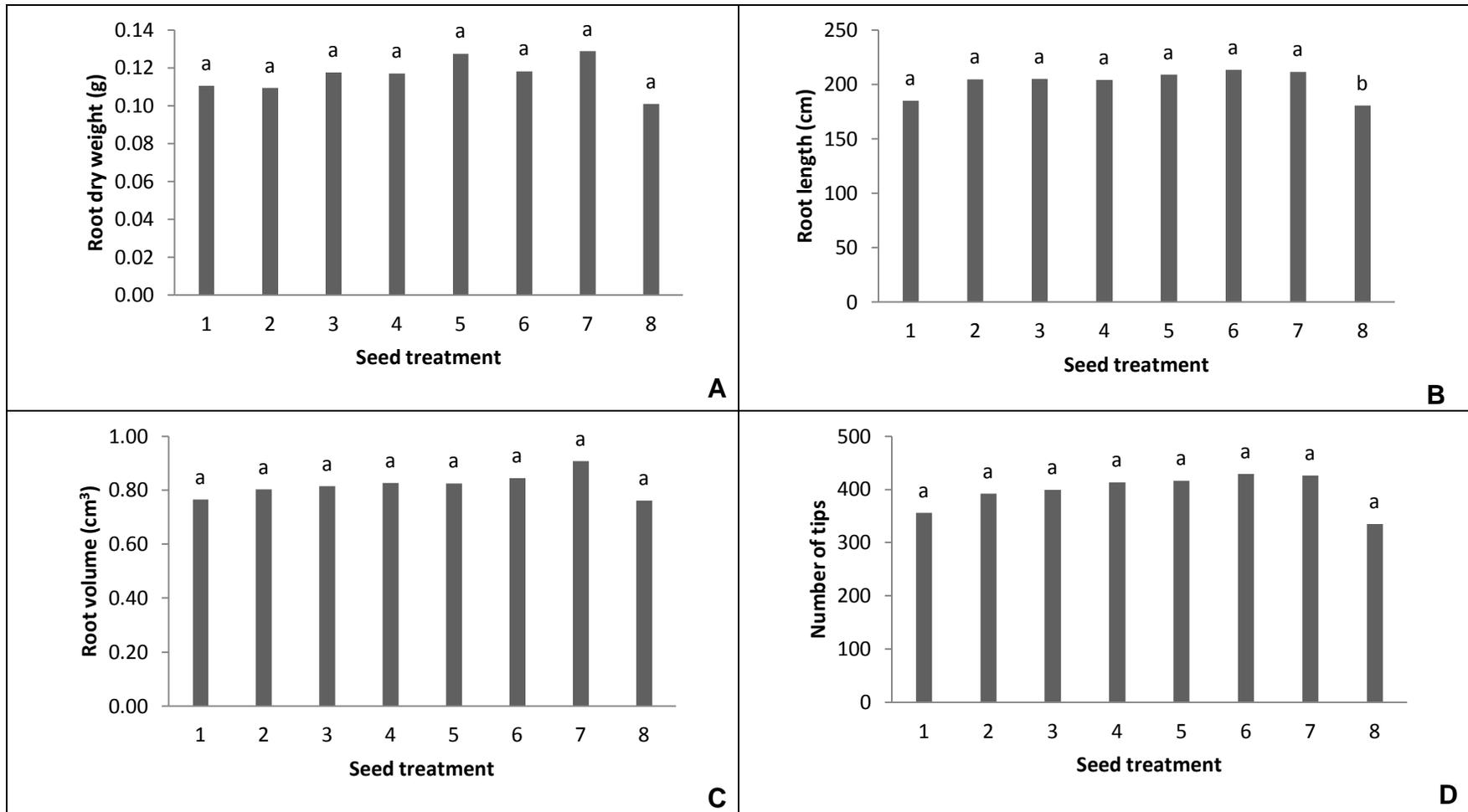


Figure 2. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Pythium ultimum* – infested sand. Treatment 1 = Fludioxo + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

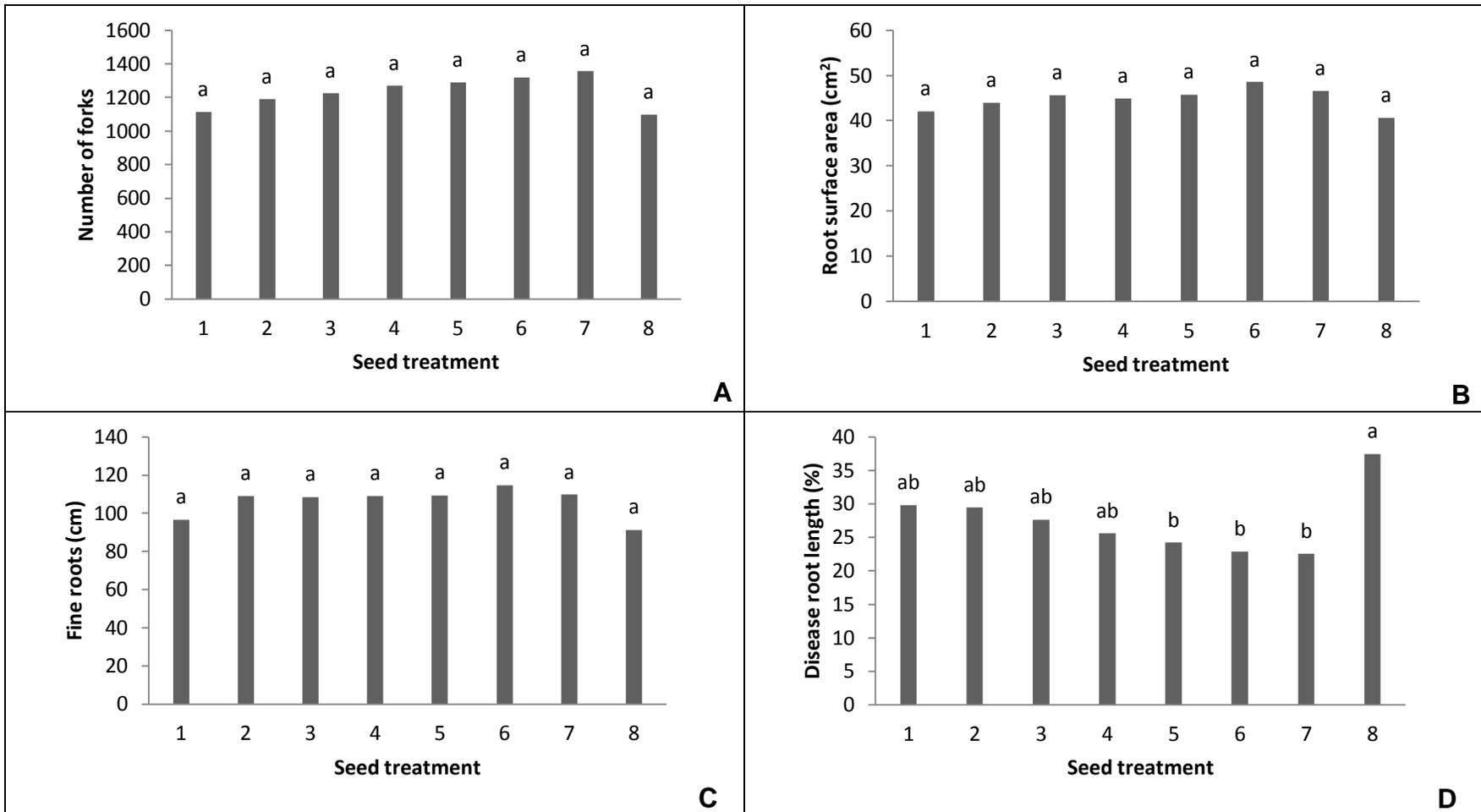


Figure 3. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Pythium ultimum* – infested sand.. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

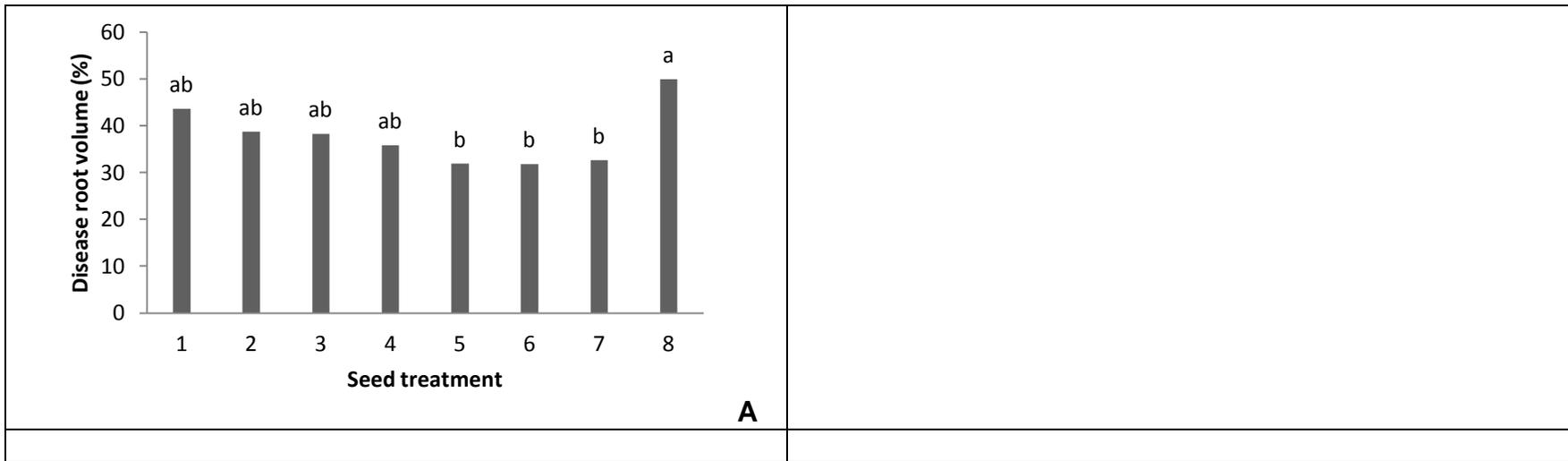


Figure 4. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Pythium ultimum* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

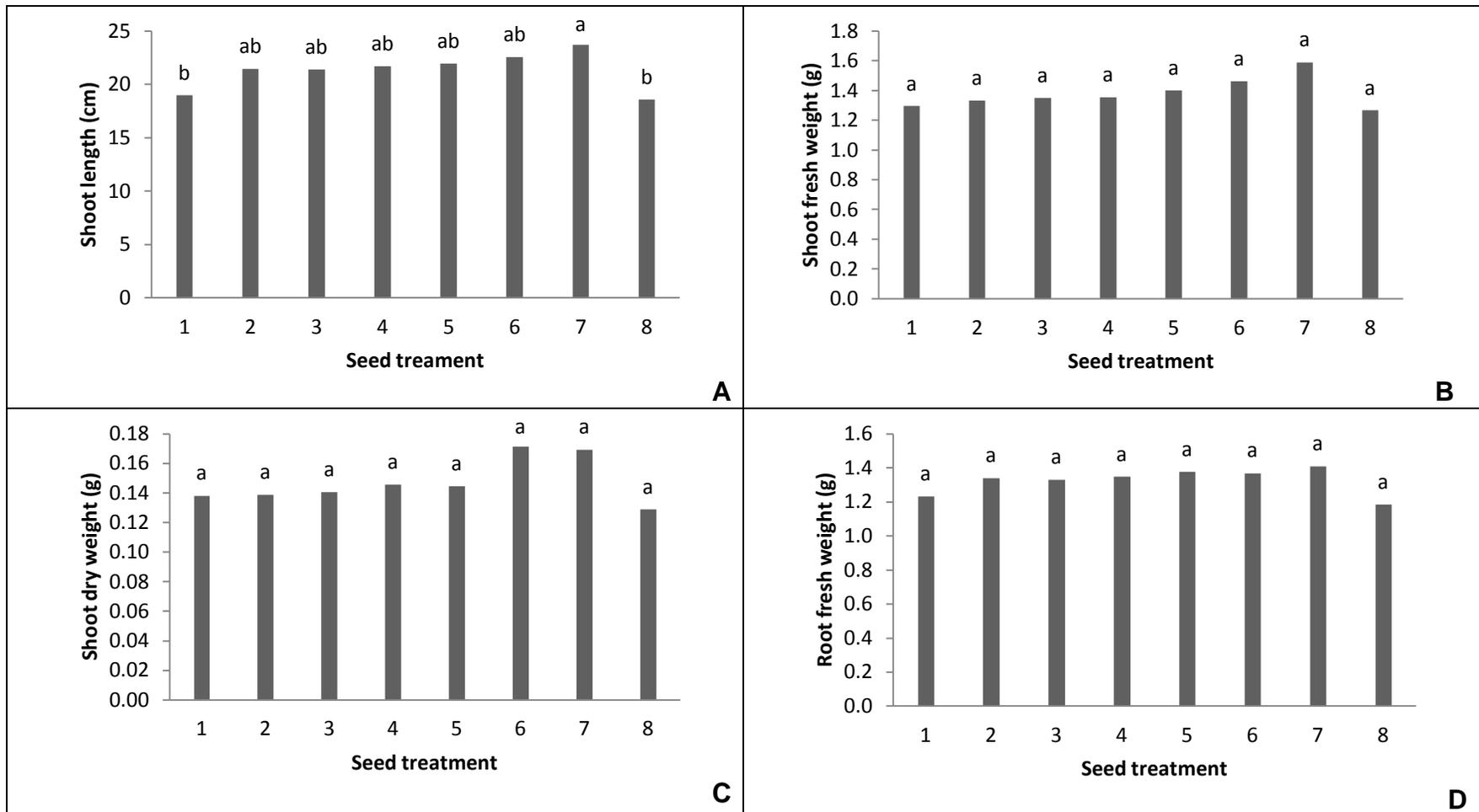


Figure 5. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Pythium ultimum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

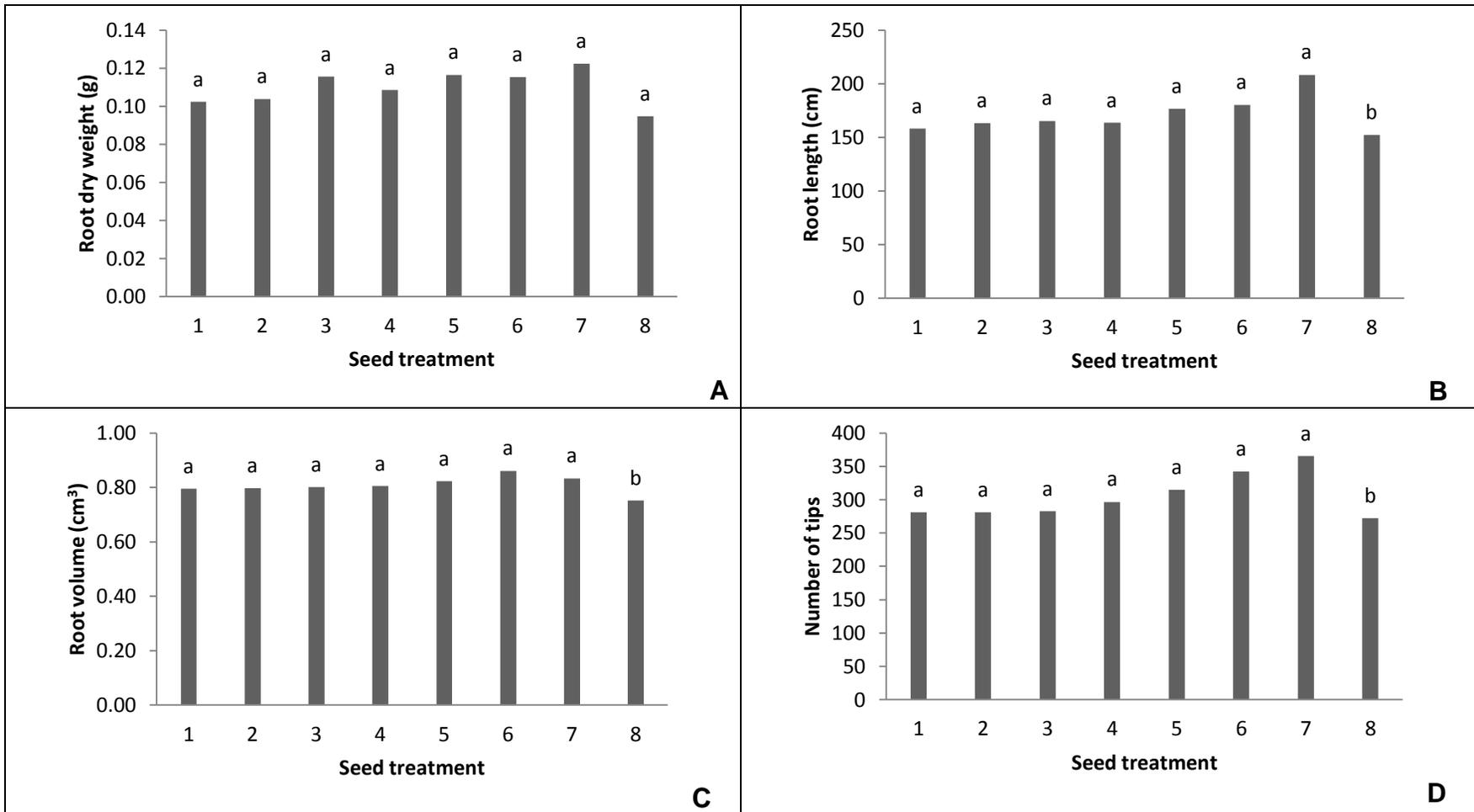


Figure 6. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Pythium ultimum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

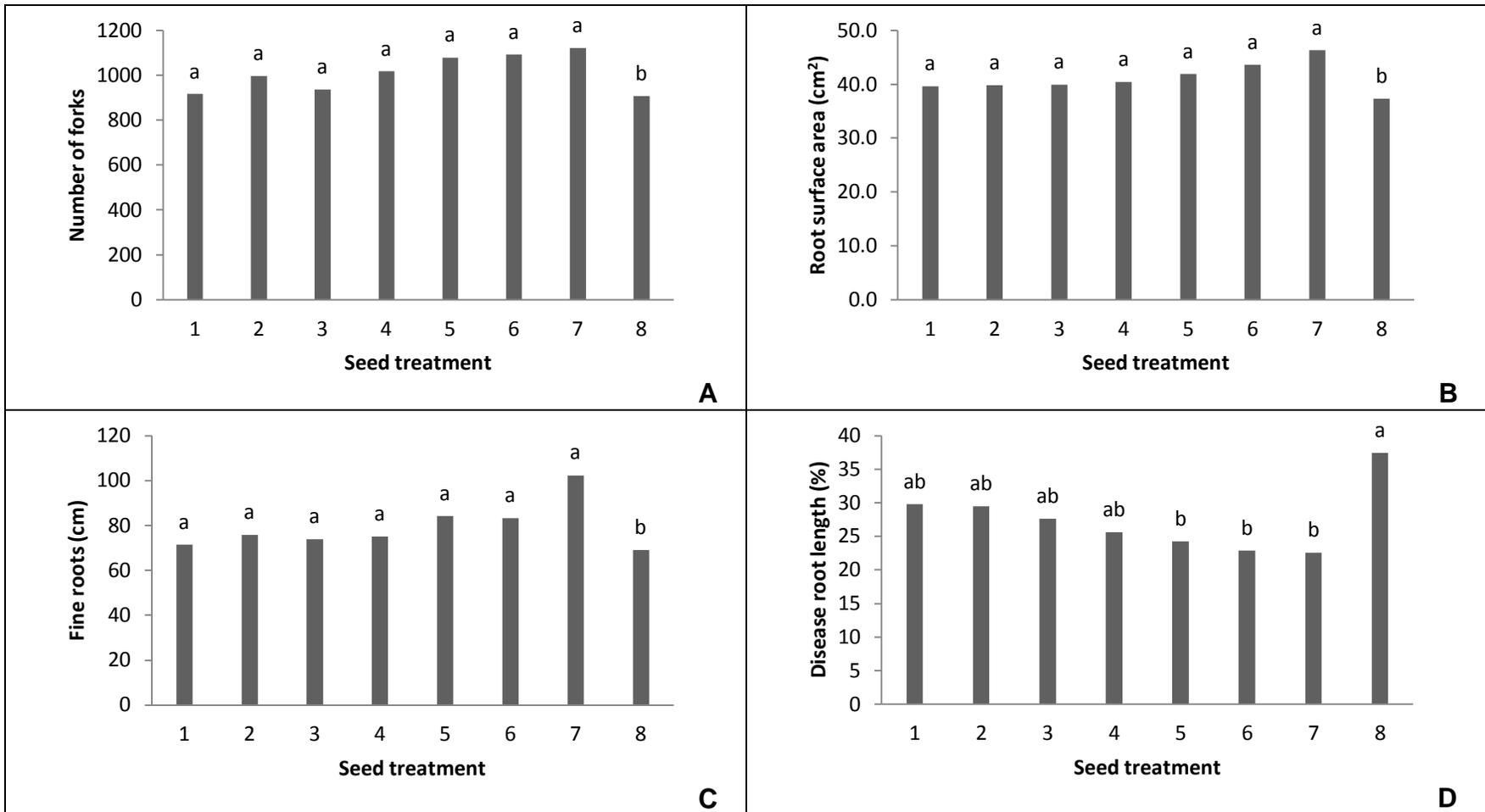


Figure 7. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Pythium ultimum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

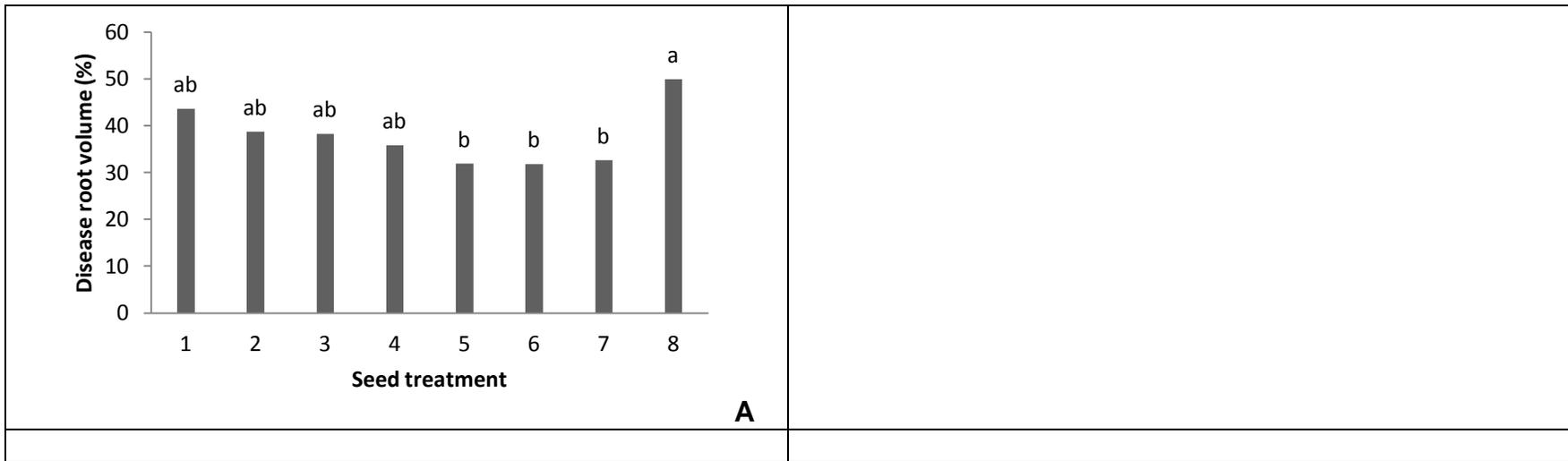


Figure 8. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Pythium ultimum*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

Table 5. *P*-values for effects of seed treatments and pathogen inoculation on seedling health variables for *Rhizoctonia solani* – inoculated plants. Analysis of variance was conducted with log transformed data except for diseased root length (%) and diseased root volume (%) which were not transformed.

Variables	Seed treatment (ST)	Fungus (F)	Nematode (N)	ST x F	ST x N	F x N	ST x F x N
Shoot length (cm)	ns	ns	ns	ns	ns	ns	ns
Shoot fresh (g)	ns	ns	0.02	ns	ns	ns	ns
Shoot dry (g)	ns	ns	ns	ns	ns	ns	ns
Root fresh (g)	ns	ns	ns	ns	ns	ns	ns
Root dry (g)	ns	ns	ns	ns	0.05	ns	ns
Root length (cm)	0.006	<.0001	ns	ns	ns	ns	ns
Root volume (cm <sup>3</sup> )	ns	<.0001	ns	ns	ns	ns	ns
Tips	0.01	<.0001	ns	ns	0.04	ns	ns
Forks	0.004	<.0001	ns	ns	0.04	ns	ns
Surface area (cm <sup>2</sup> )	0.008	<.0001	ns	ns	ns	ns	ns
Fine roots (cm)	0.01	<.0001	ns	ns	ns	ns	ns
Diseased root length (%)	ns	0.01	ns	ns	ns	ns	ns
Diseased root volume (%)	ns	0.006	ns	ns	ns	ns	ns

\* ns = not significant ( $p>0.05$ )

Table 6. *P*-values for effects of seed treatments and pathogen inoculation on nematode extraction results for *Rhizoctonia solani* experiments.

	Effects		
	Seed treatment (ST)	Fungus (F)	ST x F
Nematodes from soil	0.002	0.002	0.0006
Nematodes from roots	ns	0.03	ns

\* ns = not significant ( $p > 0.05$ )

Table 7. Number of *Pratylenchus penetrans* recovery from 100 cm<sup>3</sup> of soil and the roots therein and from 1 g of dried maize roots infested with the nematodes and / or *Rhizoctonia solani*.

Treatment	Active ingredients	Nematodes / 100 cm <sup>3</sup> soil	Nematodes / g root <sup>z</sup>
1	Fludioxonil + Mefenoxam + Azoxystrobin	3.6b	3.3
2	FMA <sup>a</sup> + 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

<sup>a</sup> FMA = fludioxonil + mefenoxam + azoxystrobin = Treatment 1

\*Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

<sup>z</sup> Non significant

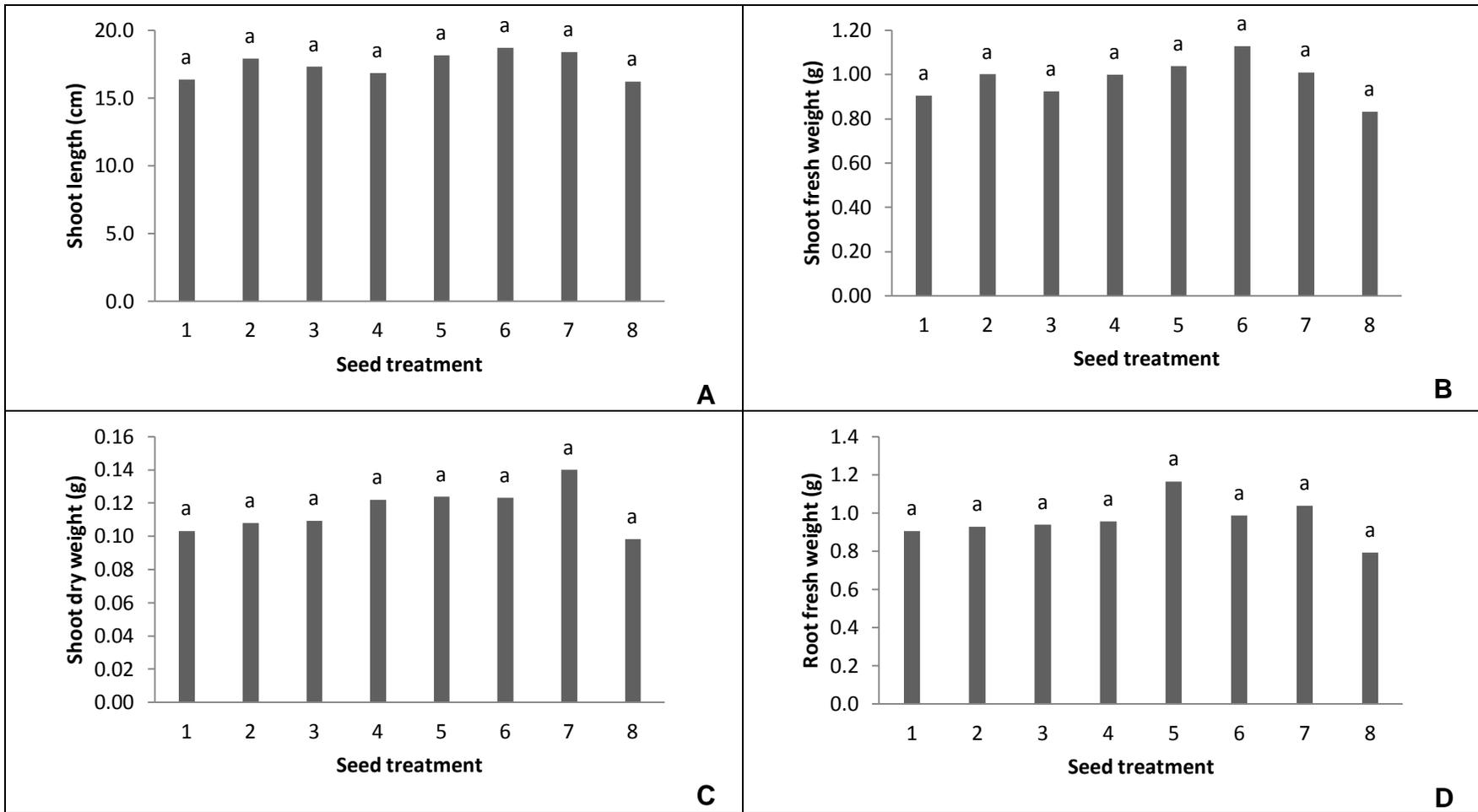


Figure 9. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Rhizoctonia solani* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

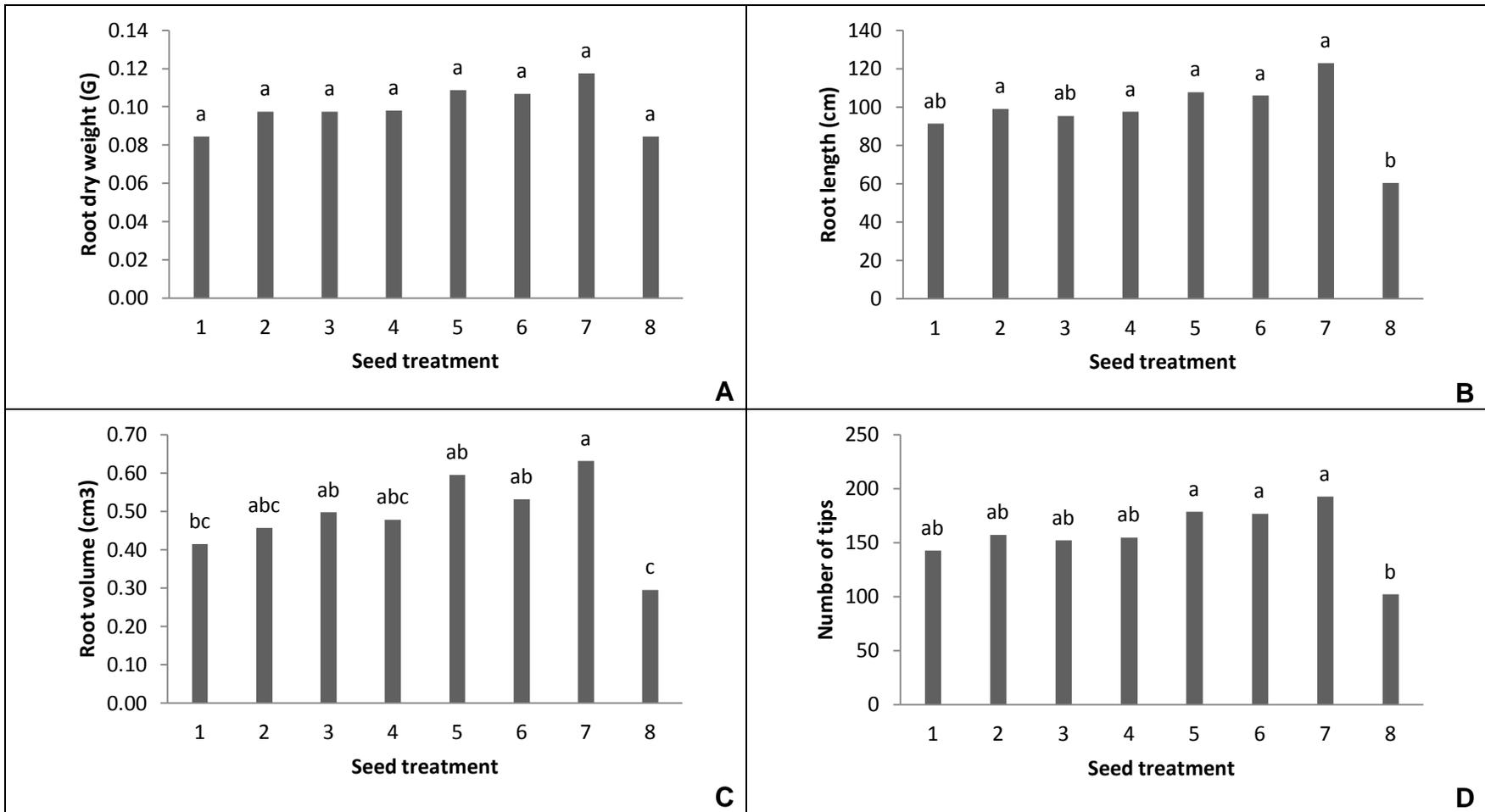


Figure 10. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Rhizoctonia solani* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

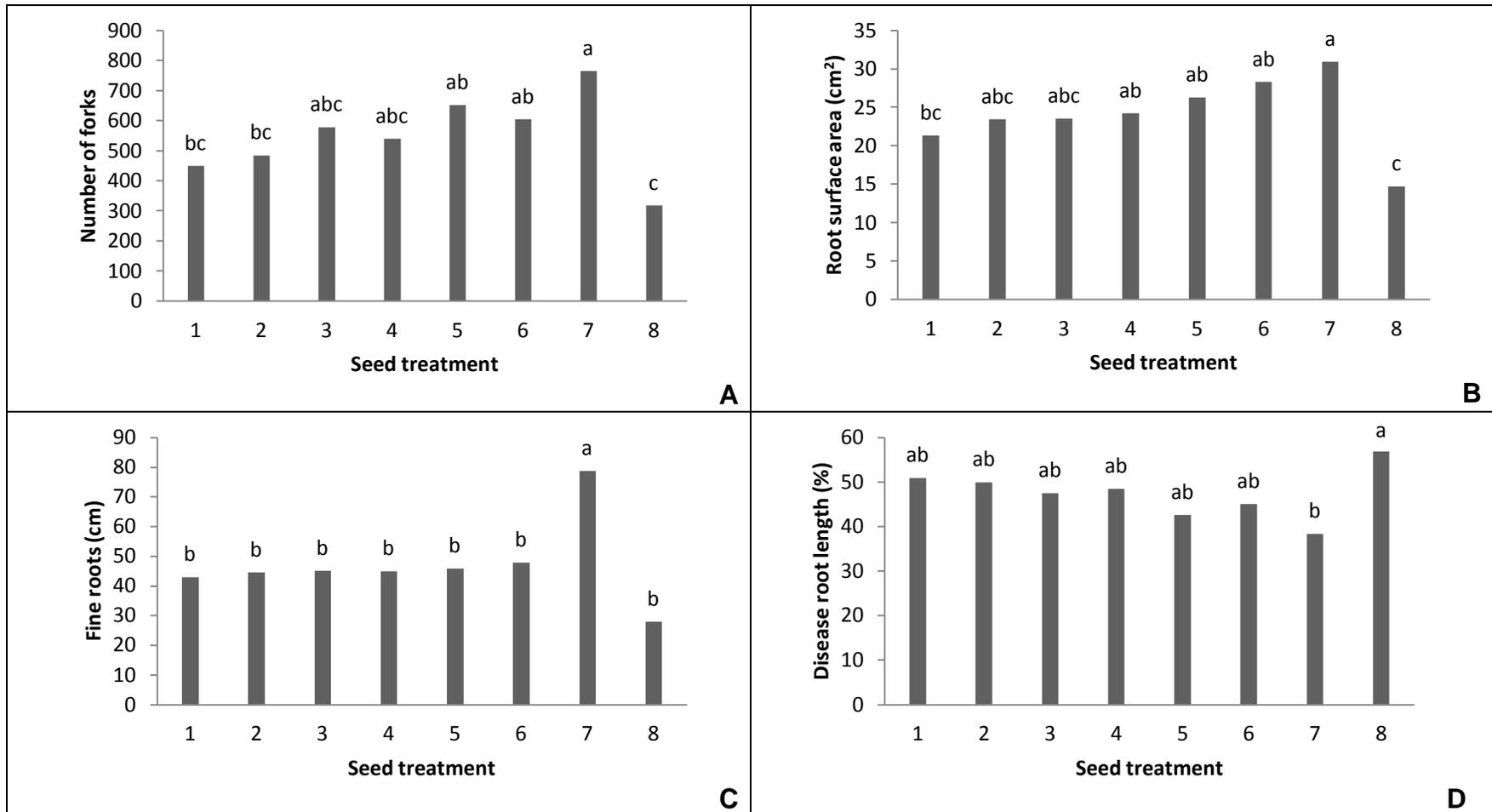


Figure 11. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Rhizoctonia solani* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

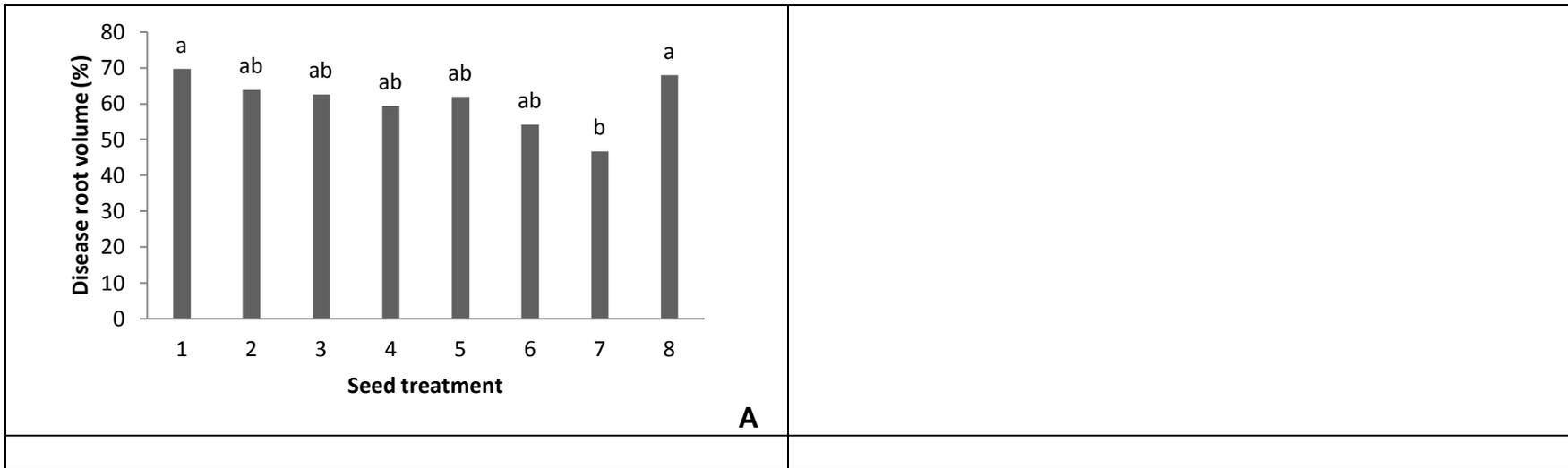


Figure 12. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Rhizoctonia solani* – infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

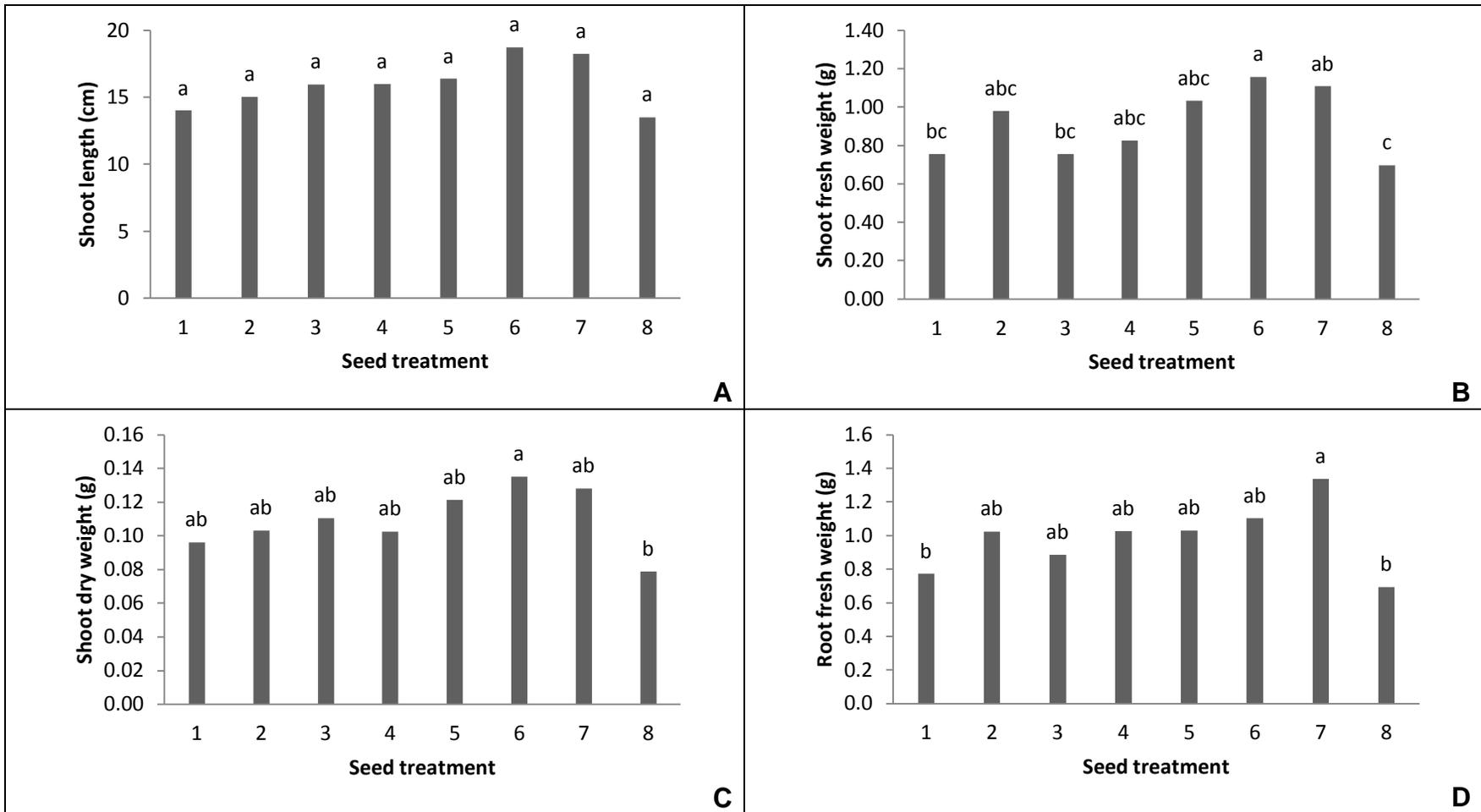


Figure 13. Shoot length means (A). Shoot fresh weight means (B). Shoot dry weight means (C). Root fresh weight (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Rhizoctonia solani*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

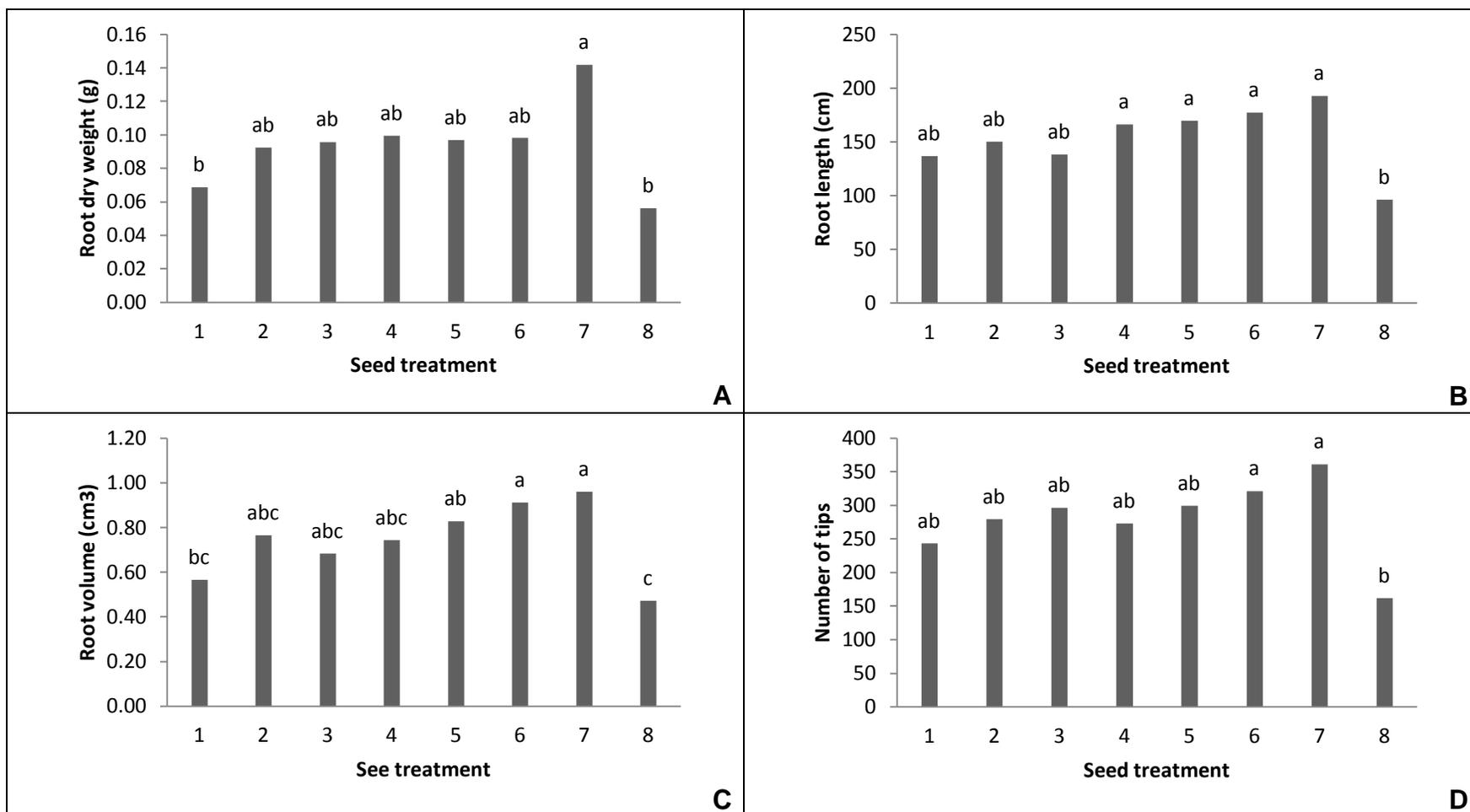


Figure 14. Root dry weight means (A). Root length (cm) means (B). Root volume (cm<sup>3</sup>) means (C). Number of tips (D) for seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Rhizoctonia solani*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

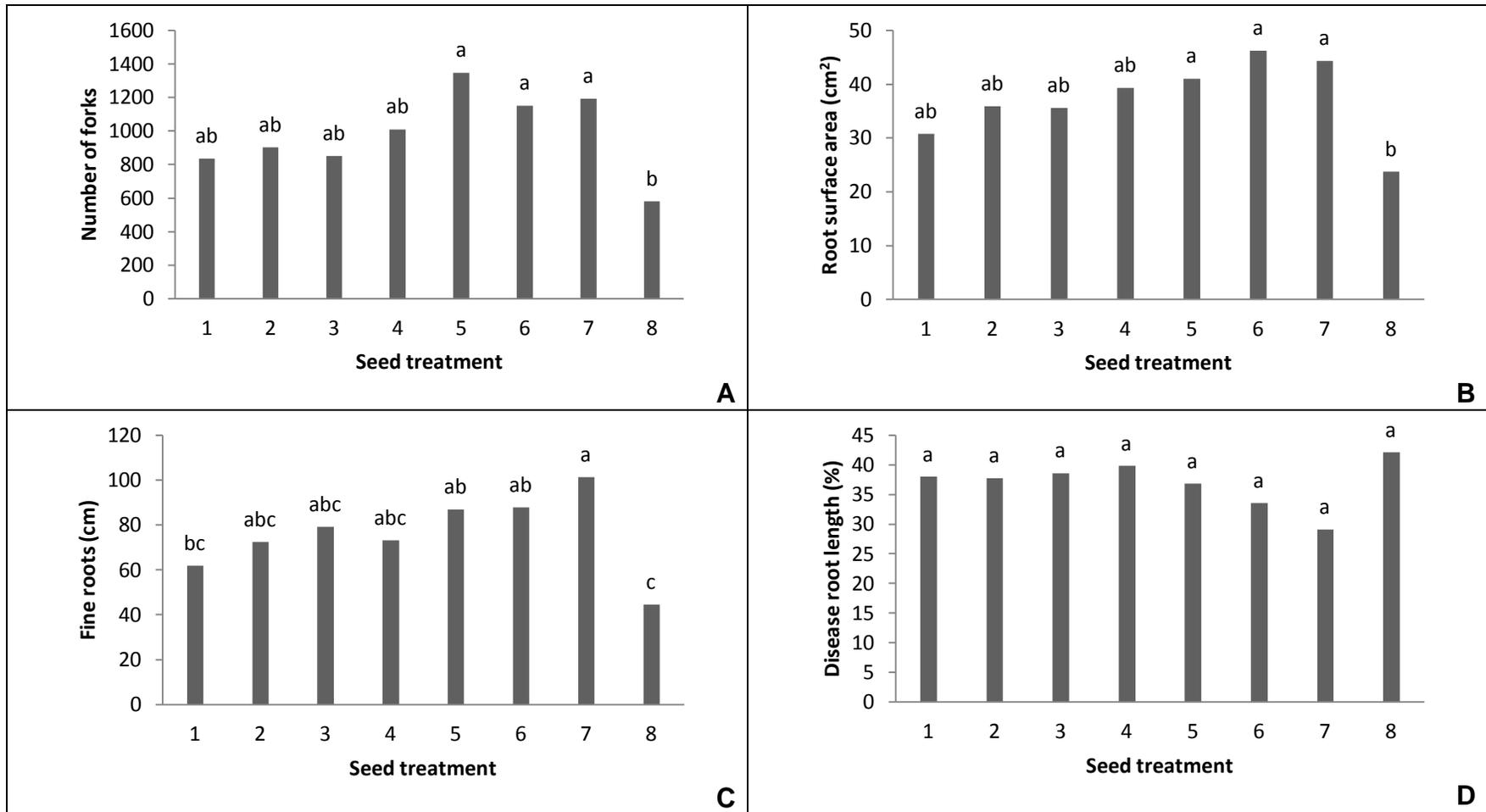


Figure 15. Number of forks means (A). Surface area (cm<sup>2</sup>) means (B). Number of fine roots means (C). Disease root length (%) (D) seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Rhizoctonia solani*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

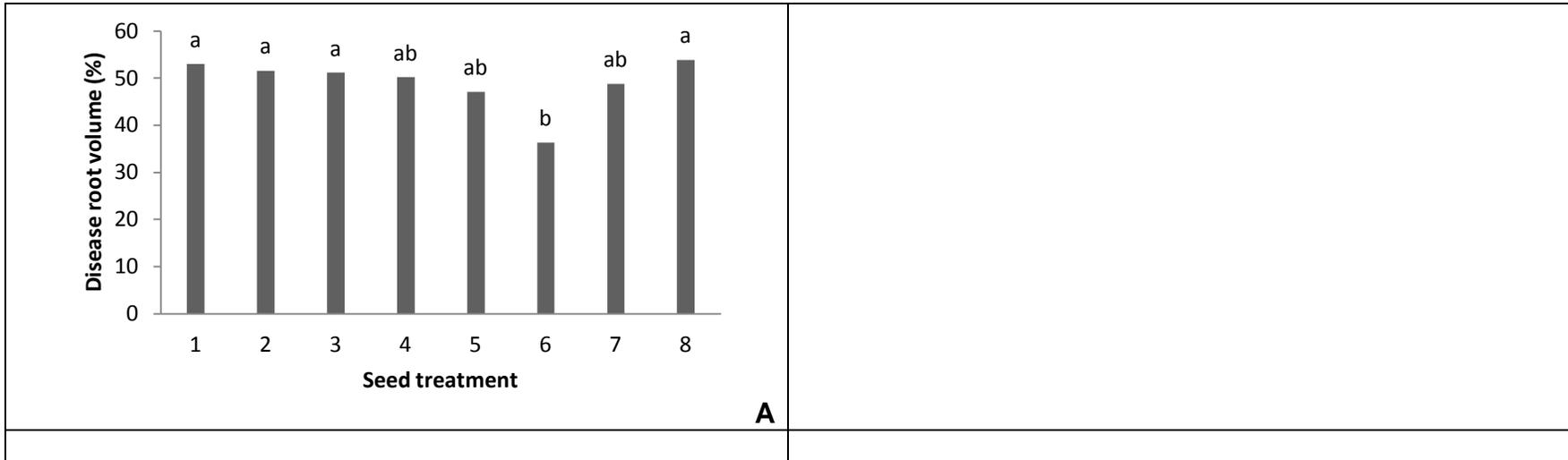


Figure 16. Disease root volume (%) (A) means seedlings from seeds treated with different seed treatment products and grown in *Pratylenchus penetrans* – infested sand. Include treatments without fungal infestation and with *Rhizoctonia solani*-infested sand. Treatment 1 = Fludioxonil + Mefenoxam + Azoxystrobin (FMA), Treatment 2 = FMA + Thiabendazole, Treatment 3 = FMA + Thiamethoxam, Treatment 4 = FMA + Thiamethoxam + Thiabendazole, Treatment 5 = FMA + Abamectin, Treatment 6 = FMA + Abamectin + Thiabendazole, Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole, Treatment 8 = Untreated). Means with the same letter are not significantly different according to the LSD test,  $\alpha = 0.05$ .

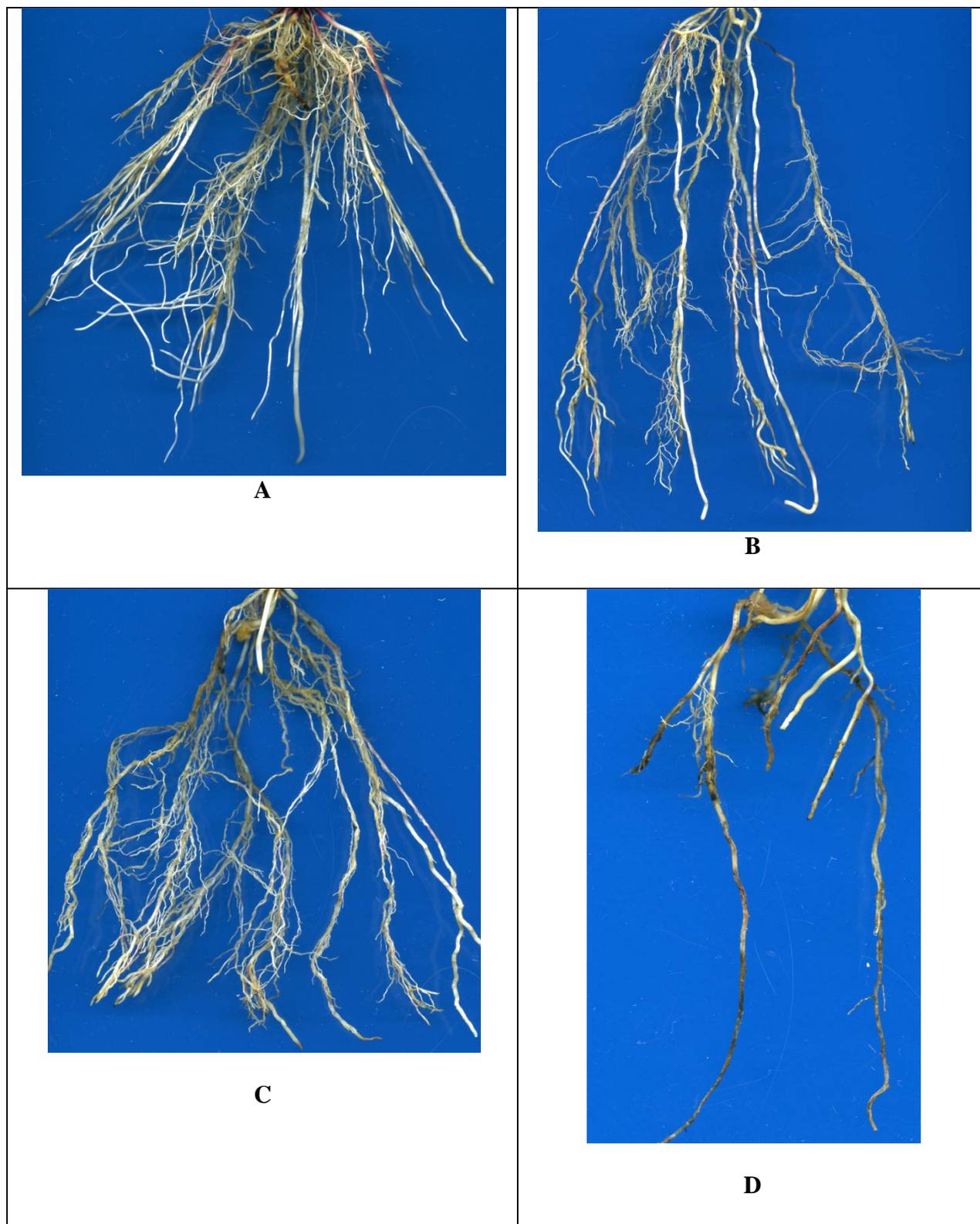


Figure 17. Pictures showing seed treatment control on maize seedlings. Treatment 5 = FMA + Abamectin (A), Treatment 6 = FMA + Abamectin + Thiabendazole (B), Treatment 7 = FMA + Abamectin + Thiamethoxam + Thiabendazole (C), Treatment 8 = Untreated (D).

## CHAPTER 4

### GENERAL CONCLUSION

The objectives of this study were to measure the effects of *Pratylenchus penetrans* infestation on seedling disease symptoms caused by fungal and Oomycete pathogens (*Fusarium graminearum*, *Fusarium verticillioides*, *Pythium ultimum* and *Rhizoctonia solani*); assess the impact of nematode control with abamectin on the above interactions and evaluate potential added seedling disease management benefit of abamectin combined with commercial fungicide seed treatment.

The results demonstrated significant effects of seed treatments on root health with interactions between fungal or Oomycete pathogens and nematodes. Seed treatments showed efficacy against fungal and nematode inoculation, improving most measures of seedling health compared to the nontreated control; mainly those seed treatment combinations 5 (FMA + abamectin), 6 (FMA + abamectin + thiabendazole) and 7 (FMA + abamectin + thiabendazole + thiamethoxan). Root structure analysis from WinRhizo showed that seed treatment significantly improved root system characteristics such as root volume, root length, number of tips, forks, surface area, fine roots and reduced diseased root length and diseased root volume. Fungal inoculation had a stronger effect compared to nematode inoculation, although diseased root length and diseased root volume were significantly affected by nematode inoculation. Indeed, seed treatment combinations with abamectin (5,6 and 7) significantly reduced diseased root length and volume when compared to the non-treated check. Data obtained in this study provide

evidence that abamectin in combination with commercial seed treatment fungicides significantly improved the protection of the maize root system against nematodes. Overall, seed treatment have shown a potential benefits of abamectin in combination with commercial seed treatment, controlling seedling disease and also nematode feeding. Besides that, Thiabendazole also presented a tendency capacity to control nematode infection.

Data obtained in this study provides enough evidence that abamectin in combination with commercial seed treatment significantly improved the protection of the maize root system against nematodes.

To our knowledge, this study is the first to evaluate and measure the potential benefits of abamectin combined with commercial fungicide seed treatment in the presence of both fungus and lesion nematodes on maize. In addition to that, our research presents novel data regarding root system characteristics in response to fungal and nematode inoculation and seed treatments, using WinRhizo (Regent Instruments Inc., Quebec, Canada).

## ACKNOWLEDGMENTS

It is a pleasure to thank those who made this thesis possible; I would like to thank all of the people who have helped and inspired me during my master's studies at Iowa State University.

First and foremost, I offer my most sincere gratitude to my major professor Dr. Gary Munkvold for his guidance and support during my thesis and for his patience and knowledge. One simply could not wish for a better or friendlier advisor. In addition, I would like to thank my POS committee members: Dr. Greg Tylka, Dr. Roger Elmore and special thanks for Dr. Alison Robertson.

I have been blessed with a culturally diverse, friendly and cheerful environment in my daily work at the Seed Science Center. In particular, I would like to thank Michele Beattie and Maria Mercedes for their friendship and help in the past three years. To all other folks, including graduate and undergraduate students from the Seed Pathology Laboratory, I appreciate all the support. Special thanks should be given to Alex Algreen who spent many hours helping me take care of the roots in the greenhouse basement.

My deepest gratitude also goes to my family and friends back in Brazil for their love and support throughout my journey at Iowa State University. I am so grateful to my father, for his support. Although he is no longer with us, he is forever remembered. I am sure he shares our joy and happiness in the heaven. I could not ask for more from my mother, as she is simply the best. Also, I would like to show my appreciation to my friends Carlos Gomes and Elcio Alves for always being so helpful.

Furthermore, I am grateful to my many friends at ISU. Special thanks for Silvina Stewart for always being a constant source of encouragement and help during my graduate studies, to Louis Thompson for his support and friendship since I started working with him, to Agustin Pagani for his help during my data analysis, to Eduarda Becerra for bringing me to ISU, and to all of those who supported me in any respect during the completion of this journey; to all of you I offer my regards and blessings. You guys rock!

Last but not least, thanks to Syngenta Crop Protection for funding this project and also special thanks to Dr. Ann MacGuidwin for nematodes source and protocols.

Finally, thanks to God for giving me strength all tests in the past three years. You have made my life more beautiful and meaningful!