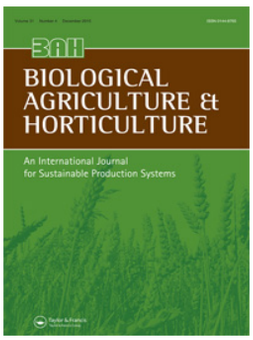

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Predatory activity of nematophagous fungi against *Panagrellus redivivus* in the soil under different plant species

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The distribution of total and nematophagous fungi and nematodes is influenced by different plant species and environmental factors. The effect of moisture content and soil organic matter (SOM) in the frequency of fungi and nematodes was examined in Eutrustox soil with lettuce (*Lactuca sativa*), banana (*Musa cavendishii*), impatiens (*Impatiens walleriana*) and bahiagrass (*Paspalum notatum*). Total fungi and nematophagous counts were made in the rhizosphere soil (RS) and non-rhizosphere soil (NRS). The fungi were isolated from each soil, and their ability to predate *Panagrellus redivivus* nematode was evaluated. The fungi frequency in the RS and NRS ranged from 6.9 to 25.6×10^5 and from 6.9 to 31.2×10^5 CFU g^{-1} soil, respectively, and nematophagous fungi accounted for 21–34% and 16–33% of total fungi, respectively. The total and nematophagous fungi counts of RS decreased in the order lettuce > banana > bahiagrass > impatiens and lettuce > impatiens > banana > bahiagrass, respectively. Both total and nematophagous fungi counts from NRS decreased in the order lettuce > banana > bahiagrass > impatiens. A positive and significant correlation showed that fungi counts were influenced by the SOM and moisture contents. The abundance of nematodes was influenced by plant species, ranging from 0 to 4.0×10^3 100 g^{-1} dry soil or roots. It can be concluded that the distribution of total and nematophagous fungi, and nematodes relates to plant species and the SOM and moisture contents.

Keywords: lettuce; nematode; non-rhizosphere soil; rhizosphere soil; soil filamentous fungi

Introduction

Nematophagous fungi are distributed in all regions of the world, having been found in agricultural, forest and garden soils (Olivares-Bernabeu et al. 2003; Acevedo-Ramírez et al. 2011; Alfaro Gutiérrez et al. 2011). Numerous losses to agricultural production have been attributed to nematodes by interfering with the dynamics of physiological processes of plants (Mattos et al. 2006). Although many fungal predators of nematodes have been isolated and identified, much about the growth of these fungi in the soil (Cardoso et al. 2009) and their predatory activity is not yet known.

The frequency of nematophagous fungi can be affected by numerous environmental, nutritional and physiological properties of the soil. Jaffee (2002) reported that the addition of vine leaves as a source of organic matter increased the population and predation activity of the fungi *Arthrobotrys oligospora* and *A. eudermata* to *Dactylellina haptotyla*. Different sources of organic matter showed differential effects in the control of the

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nematode *Meloidogyne mayaguensis* in tomato roots by *A. oligospora* (Duponnois et al. 2001). In addition, the influence of environmental conditions on the frequency and diversity of nematophagous fungi has been reported. *Arthrobotrys* and *Monacrosporium* were found in the rhizospheres of banana because this plant provides optimum conditions of moisture, soil temperature and nutrient supply for the development of the fungi (Ribeiro et al. 2003). Factors such as pH, temperature, light intensity, and carbon and nitrogen sources influenced the nematophagous fungi growth (Hasanzadeh et al. 2012); however, no relationship among the occurrence, origin and distribution of *Arthrobotrys* from different Brazilian localities and different crops was found (Oliveira et al. 2002). According to Nordbring-Hertz et al. (2006), ecological factors that influence the nematophagous fungi have been partially identified. Among the chemical characteristics of the soil, the contents of organic matter and moisture have been identified as the key factors that influence the structure and number of microorganisms (Lauber et al. 2008; Swer et al. 2011; Garcia & Nahas 2012).

The region of the soil under the direct influence of plant roots is referred to as the rhizosphere and is characterized by exudation of a wide variety of compounds and their influence on microorganisms and nutrient cycling (Walker et al. 2003). The composition and amount of exudates depend on the plant species, stage of development, intensity of photosynthesis and nitrogen fertilization (Kuzyakov 2002; Houlden et al. 2008). Therefore, different plant species may provide different rhizospheric effects, which affect the composition and diversity of fungal populations (Broeckling et al. 2008). As the microorganisms depend on a source of carbon and energy that can be found in the rhizosphere, their growth is conditioned on plant species investigated. The effect of the rhizosphere can be further demonstrated when compared with the fraction of non-rhizospheric soil. It has been reported that the population count and the number of genera of fungi from the rhizosphere soil (RS) were higher than from non-rhizosphere soil (NRS) (El-Hissy et al. 1980; Nordbring-Hertz et al. 2006).

It should be considered that nematodes are a food source for nematophagous fungi (Olivares-Bernabeu et al. 2003), producing different types of traps that capture nematodes (Barron 2003; Yang et al. 2003; Hsueh et al. 2013). In the soil, where stressful nutritional conditions for fungal development can prevail, the ability to prey on nematodes gives them additional survival advantages. Some fungal species develop structures that capture nematodes as a result of external stimuli, whereas others develop structures spontaneously, being more dependent on nematodes as a nutrient source.

Therefore, this study was conducted to investigate the frequency of total and nematophagous fungi in the soil under different species of horticultural (lettuce), ornamental (impatiens), fruit (banana) and grass (bahiagrass) plants. The frequency and genera of nematodes were evaluated in order to establish greater understanding of the prevalence of nematophagous fungi in the soil below these species of plants.

Material and methods

Sites of collection of soil and root samples

For NRS, Eutrustox soil samples from lettuce (*Lactuca sativa* L.), banana (*Musa cavendishii* Lambert ex Paxton), impatiens (*Impatiens* sp.) and bahiagrass (*Paspalum notatum* Flüggé) were collected at a 0–15 cm depth from Jaboticabal County, SP, Brazil. The areas of bahiagrass and banana were approximately 0.5 ha and 1 ha in size, respectively. The banana crop, which was established 12 years ago, was fertilized only in this plantation with 160 kg N (as urea) ha^{-1}, 40 kg P_2O_5 (as triple superphosphate) ha^{-1}

and 40 kg K_2O (as KCl) ha^{-1}. Lettuce and impatiens were grown in beds of approximately 1 m × 10 m and 2 m × 3 m, respectively. Only lettuce was fertilized with 10 ton ha^{-1} chicken manure. For each plot, five samples were taken randomly, and each sample consisted of four replicates. A block of soil containing plant roots was collected for RS. This block was broken leaving the roots with adhering soil.

The moisture and organic matter contents of the soil samples were determined after drying the samples at a temperature of 105°C and incineration of the soil at 550°C, respectively. The moisture content of the roots was measured after drying in an oven with air circulation at temperature of 65°C for 2 days.

Frequency of total and nematophagous fungi

The soil samples were serially diluted from 10^{-2} down to 10^{-3} and inoculated onto a solid culture medium (Martin 1950), pH 5.6 with 60 μg penicillin ml^{-1}, 40 μg streptomycin ml^{-1} and 70 μg rose Bengal ml^{-1} and incubated at 30°C for 72 h. Counts of the number of colony-forming units (CFU) of fungi were made according to method of Vieira and Nahas (2005). The colonies of fungi with different morphological characteristics were isolated and incubated for 7 days on Sabouraud agar medium. Cultures were renewed every 30 days.

Nematophagous activity of fungal isolates

TTotal 673 strains of fungi were inoculated in 6 cm diameter Petri dishes containing water agar (2% agar, w/v) and were incubated for 4 days. Subsequently, 100 *Panagrellus redivivus* nematodes were placed in each plate and incubated at 30°C. Every 24 h, dead nematodes were counted under a stereomicroscope at a maximum of 40 × zoom. As a control, plates were inoculated only with nematodes or nematodes and the fungus *A. oligospora*. The number of *P. redivivus* was measured according to the Heintz (1978) method. Briefly, 1.0 ml of an aqueous suspension with approximately 1000 *P. redivivus* nematodes was inoculated into each sterile polystyrene Petri dish containing fine oat flakes and water in a 1:1 ratio stored at room temperature in the dark and subcultured every 15 days.

Population density and identification of nematodes

Samples of 100 or 10 g of ground roots were used for the extraction of nematodes by the Jenkins (1964) and Coolen and D'Herde (1972) methods, respectively. The count of the number of extracted nematodes was performed using Peters blade under a microscope. The genera of nematodes were identified by examination of samples of the suspensions on slides under a microscope based on morphological and morphometric characteristics. The counts of fungi and nematodes have been expressed per gram of dry soil.

Statistical analysis

A completely randomized design with four treatments and five replications was used. Analysis of variance was performed using the SAS (1990) program. The mean values were compared by the Tukey's test at $p \leq 0.05$. The fungi counts were transformed into $\log(x + 1)$ where $x =$ number of CFU g^{-1} dry soil. Correlation analysis (r) was performed to examine the relationships between soil variables.

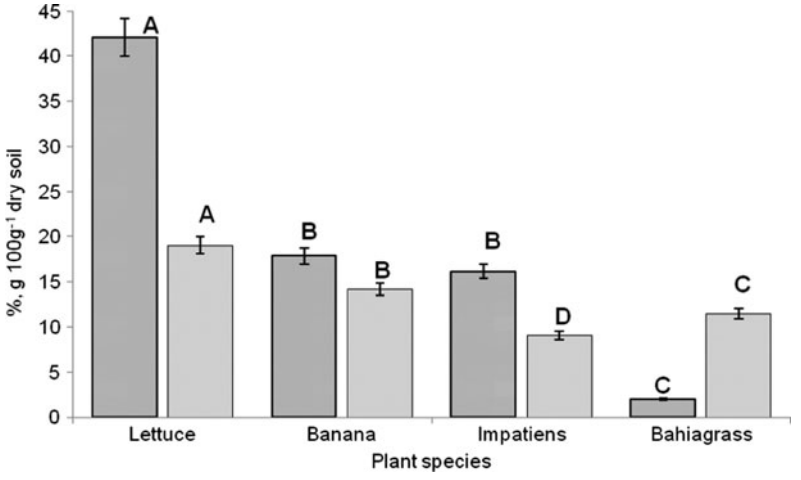

Figure 1. Moisture (▨) and organic matter (□) contents of the soils under different plant species. Means followed by the same letter do not differ by Tukey's test at $p < 0.05$. Vertical bars indicate the standard deviation (SD).

Results and discussion

The moisture and soil organic matter (SOM) in lettuce soil were, respectively, 2.4–20.8 and 1.3–2.1 times higher than those found in other soils (Figure 1). The total fungi counts ranged from 6.9×10^5 to 31.2×10^5 CFU g^{-1} in NRS and from 6.9×10^5 to 25.6×10^5 CFU g^{-1} in RS (Figure 2). The CFU number in the lettuce soil was 3.3–4.5 times higher in NRS and 1.9–3.7 times higher in RS than the CFU found in the other soils. The number of fungi with nematophagous activity ranged from 1.1×10^5 to 6.8×10^5 CFU g^{-1} in NRS and 2.1×10^5 to 8.8×10^5 CFU g^{-1} in RS (Figure 3). As with the total fungi counts, nematophagous fungi also predominated in the lettuce soil and were higher by 2.2–6.0 times in the NRS and 2.1–4.3 times in the RS when compared with CFU of other plant species.

Several authors have reported that the distribution of fungi has been related to the type of plant (Barroti & Nahas 2000; Lauber et al. 2008; Val-Moraes et al. 2013). According to these authors, there was variation in the CFU numbers of both total fungi and nematophagous fungi in the soil under different plants. Several studies have shown that environmental factors such as soil pH, moisture and organic matter influence the counts of total (Singh et al. 2013) and nematophagous (Gray 1988; Hasanzadeh et al. 2012) fungi. Table 1 shows that the counts of total and nematophagous fungi in both NRS and RS were significantly correlated with the SOM and moisture contents.

The nematophagous fungi counts in the RS were 29% (lettuce) to 277% (impatiens) higher than in the NRS; in contrast, CFU counts in banana and bahiagrass soil were lower by 21–35%, respectively (Figure 3). The relationship between the total number of fungi in the RS and NRS varied from 0.8 to 2.01 and nematophagous fungi from 0.7 to 3.8. This study corroborated the previous data (Pandey & Palni 2007) that showed a ratio from 0.8 to 3.4 in 10 species of plants in Uttaranchal, India. The greater growth of fungi in RS may be due to the presence of exudates, secretions, lysates of plants and slimes with the release of carboxylic acids, carbohydrates, amino acids and amides that are used as a substrate (Canbolat et al. 2006). The exudates released by each plant species can be quite specific (Jones et al. 2004) and, consequently, exert different influences on the growth of fungi.

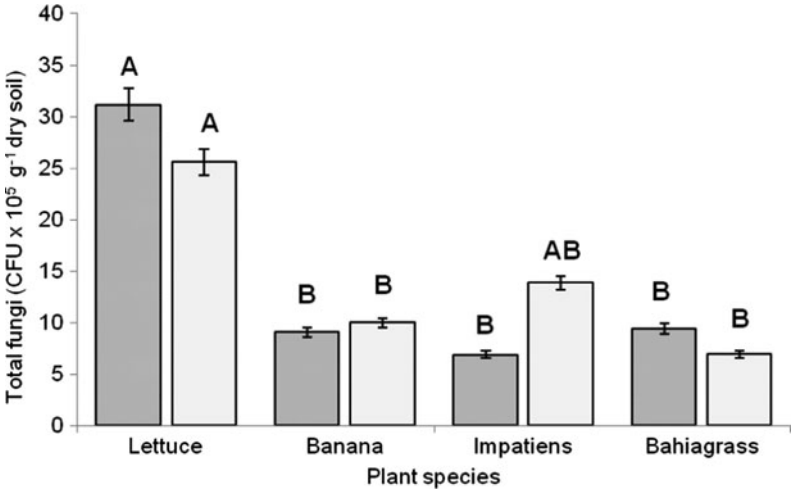

Figure 2. Total fungi from non-rhizosphere (▨) and rhizosphere (□) soils under different plant species. Means followed by the same letter do not differ by Tukey's test at $p < 0.05$. Vertical bars indicate the standard deviation (SD).

The expressive count of nematophagous fungi in the rhizosphere found in this study may have significant importance in the control of nematodes in the vicinity of plant roots (Siddiqui & Mahmood 1996; Nordbring-Hertz et al. 2006).

Of the total fungal isolates, 21–34% of those found in RS and 16–33% in NRS (Figure 3) showed activity against *P. redivivus*. These percentages were higher than those found in 150 samples of grassland soils and 138 sheep faecal samples, from which only 1.5% of the total fungal isolates had nematophagous activity against *Haemonchus contortus* (Ghahfarokhi et al. 2004). In addition, approximately 13% of soil fungi in Spain were found to be parasitic towards nematodes (Olivares-Bernabeu & López-Llorca 2002).

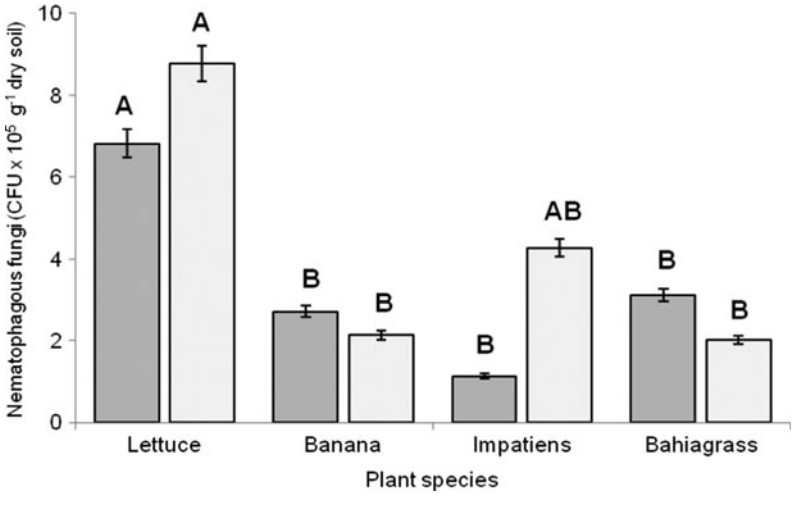

Figure 3. Nematophagous fungi from non-rhizosphere (▨) and rhizosphere (□) soils under different plant species. Means followed by the same letter do not differ by Tukey's test at $p < 0.05$. Vertical bars indicate the standard deviation (SD).

Table 1. Correlation between fungi counts and chemical properties of soil under different plants species and management regimes.

	TFR	NFNR	NFR	OM	Moisture
TFNR	0.74***	0.90***	0.80***	0.88***	0.86***
TFR	–	0.48*	0.82***	0.56*	0.77***
NFNR	–	–	0.62**	0.90***	0.72***
NFR	–	–	–	0.63**	0.80***
OM	–	–	–	–	0.80***

TF, total fungi; NF, nematophagous fungi; R, rhizosphere soil; NR, non-rhizospheric soil; OM, soil organic matter; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Calculated with the means of five replicates of each treatment.

Different numbers of nematodes were found, ranging from 0 to 4.04×10^3 100 g^{-1} soil or roots depending of plant species (Table 2). These results are in agreement with those in the literature that have shown the influence of different vegetation and organic matter concentration on the frequency of nematodes (Yeates 1979; Asmus et al. 2008; Hu & Qi 2010). Lower frequency of total nematodes (soil + roots) was found in lettuce and bahiagrass than in impatiens and banana. The total number of nematodes found ranged from 3.89×10^2 nematodes 100 g^{-1} soil to 4.47×10^3 nematodes 100 g^{-1} roots, corresponding to, on average, 11.5 times greater number of nematodes in the roots than in the soil. The frequency of nematodes in the soil obtained in this study was lower than that found in the soil in Sweden (170–1030 nematodes 100 g^{-1} soil) (Persmark et al. 1996) and in Kenya (12–584 nematodes 100 g^{-1} soil) under lettuce (Maina et al. 2010). The conditions used in this work and the type of soil must have influenced these results. However, the results obtained in this study were higher than those found by Maina et al. (2010), who reported 2–60 nematodes 10 g^{-1} roots.

The frequency of nematodes decreased in the order: *Meloidogyne* > *Helicotylenchus* > *Rotylenchus* > *Paratylenchus* > *Pratylenchus* > *Tylenchus*. The higher frequency of nematophagous fungi found in the soil under lettuce (Figure 2) can be related to the highest concentration of SOM and moisture. However, in the lettuce soil, the lowest number of nematodes was found (Table 2), which apparently contradicts the results reported in the literature. However, it must be considered that fungi have different relationships, saprophytic or parasitic, with nematodes. The fungi isolated from the soil in this study can be considered optional parasites (Barron 2003), and, according to Persmark

Table 2. Genera and numbers of nematodes found in the roots and soil from different plant species.

	Lettuce		Banana		Impatiens		Bahiagrass	
	Soil	Roots	Soil	Roots	Soil	Roots	Soil	Roots
Genus	Nematodes 100 g^{-1} dry soil or 100 g^{-1} dry roots							
Meloidogyne	ND	ND	17 B	ND	25 A	4036 a	1 C	6 b
Paratylenchus	ND	ND	ND	ND	ND	ND	3	177
Pratylenchus	ND	ND	1 A	ND	1 A	ND	ND	23
Rotylenchus	3 C	18 a	132 A	ND	55 B	ND	ND	23 a
Helicotylenchus	7 C	ND	107 A	155 a	2 C	34 b	29 B	2 c
Tylenchus	1	ND	ND	ND	5	ND	ND	ND
Total	11	18	257	155	88	4070	33	231

ND, not detected. Means within genera followed by the same letter, uppercase (soil) and lowercase (roots) do not differ by Tukey's test at $p < 0.05$.

et al. (1996), no correlation between these fungi and nematodes was found. Another possibility is the stimulation of the activity of microorganisms that control nematodes reducing their number (Akhtar & Malik 2000), as observed in the lettuce soil. Thus, the highest counts of nematodes, especially *Meloidogyne*, found in the impatiens soil could be due to lower counts of total fungi and nematophagous observed in this soil.

It can be concluded from this study that there was a high proportion of nematophagous fungi (24–31% of total fungi) and strong evidence of the influence of plant species and chemical variables on the total and nematophagous fungi counts. The results obtained in the lettuce soil showed that soil fertility, resulting from fertilization with chicken manure, and moisture and SOM contents could have increased the total number of nematophagous fungi and caused, as a result, a reduction of the number of nematodes.

Disclosure statement

No potential conflict of interest was reported by the authors.

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