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Soil Nematodes of Brassica *rapa*: Influence of Temperature and pH

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Abstract

An investigation of the soil nematodes associated with *Brassica rapa* and the effects of temperature and pH on their populations was carried out. The nematodes recovered were categorized into three ecologically functional groups; Nitrogen Releasing Nematodes (NRN, bacterial- and fungal- feeders), Plant-Parasitic Nematodes (PPN), and Pest Suppressor Nematodes (PSN, Carnivorous or predatory (Ca) and Omnivorous (Om), nematodes). While over 67% of the NRN were bacterial feeding nematodes (Ba_2), dominated by *Plectus* and *Acrobeloides;* over 65% of the PPN were contributed by the Pl_3 nematodes dominated by *Helicotylenchus mucronatus* and *Rotylenchus buxophilus*; and the PSN were dominated (over 63%) by the omnivores (Om_4; Mesodorylaimus and Dorylaimus). Populations of all the nematode categories recovered fluctuated significantly ($P < 0.01$) during the sampling period. All the nematode groups were similarly weakly negatively correlated with pH and temperature. Approximately 1000 *H. mucronatus* nematodes/100 mL of soil was recorded. This is 10x the value for which the management of PPN is recommended. The entire soil nematode food web structure was represented in the nematodes recovered, all groups of nematodes responded similarly to environmental changes, all groups of nematodes tended to be acidophilic and thermophobic, and the PPN could potentially be a threat for profitable *B. rapa* production.

Key words: Soil nematodes; Ecological function; *Brassica rapa*; Acidophilic; Thermophobic

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1. INTRODUCTION

1.1 Nematodes of Brassica *rapa*

Despite the health and nutritional benefits derived from turnips (*Brassica rapa*), these crops still have their share of pest species. In previous studies some of the major and most frequently encountered plant-parasitic nematode genera associated with *Brassica spp*., were *Pratylenchus spp*., *Helicotylenchus spp*., *Heterodera spp*., *Tylenchorynchus sp*., *Ditylenchus sp.*, *Meloidogyne spp.*, and *Paratylenchus spp.* (Mennan & Handoo, 2006; Holgado et al., 2008; Knight et al., 1997). While there is some literature on the plant-parasitic nematodes of *Brassica spp*. in certain parts of the world because of their destructive potential, there is paucity of literature on the beneficial-free-living soil nematodes associated with this important vegetable.

Objective: Plant-parasitic nematodes have a stylet used to pierce plant tissues and are major biotic pests of crops and other plants. Their destructiveness to plants could range from slight to total loss and their microscopic nature hides their activities to the detriment of the grower. On the other hand, non-stylet bearing free-living nematodes are agroecologically desirable (Ferris & Matute, 2003; Matute et al. 2013).This investigation seeks to document for the first time in the study area, the complete nematode community structure of *B. rapa* according to trophic groups, their abundances, and the effect of sampling time on their populations.

1.2 Soil Nematode Populations and pH

pH is an important physico-chemical soil factor that is expected to have a bearing on the structure and abundance of microbial soil communities. In a long-term study by Korthals et al. (1996), they reported that combinations of high Copper and low pH significantly reduced the number of bacterial-feeding nematodes, whereas the number of hyphal-feeding (fungivorous) nematodes increased. Their results suggested that the nematode community structure was indirectly affected by Copper and pH via other components of the soil food web; In a mesocosm experiment by Raty and Huhta (2003), they reported that nematodes were significantly more abundant in limed than unlimed humus soil in the absence of earthworms (also Russom et al., 1993); In another study conducted in Iowa and Wisconsin (USA), it was demonstrated that soybean cyst nematode population densities are often highest in soils of pH 7.0 or higher as compared to areas of soil pH 5.9-6.5 (Warner, 2009); In a related study, free-living stages of *Steinernema kraussei* (Rhabditida), an endoparasite of *Cephalcia abietis* L (Hymenoptera), were exposed to different soil conditions when searching for host nymphs. In their field and laboratory studies, Fisher and Fuhrer (1990) showed that acidic soil with pH levels below 4.0 may limit the nematodes' host-finding ability; In a study by Burns (1971), 'Amsoy' soybeans were grown for 2 months in non-sterilized Jacson silt loam amended to pH 4.0, 6.0, and 8.0. Nematodes were extracted biweekly from soil and roots. The greatest numbers of *Pratylenchus alleni* colonized soybean roots at pH 6.0. *Hoplolaimus galeatus* and members of the Tylenchinae-Psilenchinae survived best at pH 6.0, while members of the Dorylaimoidea were greatest at both pH 6.0 and 8.0. The non-stylet nematodes were recovered in greater numbers from pH 8.0 soils. Nematodes were fewest at pH 4.0 in the soil. It is thus evident that some nematode groups thrive best in acidic conditions while others are favored by alkaline soil conditions.

Objective: This investigation seeks to elucidate the possible effect that pH has on the different soil nematode trophic groups associated with *B. rapa* in the study area.

1.3 Temperature and Nematode Populations

Temperature is an important soil factor capable of regulating microbial activity and shaping the soil microbial community. Not much exist in terms of literature on how temperature affects the most abundant invertebrate group (i.e. nematodes) of the soil microorganisms, in a natural ecological setting. In a laboratory experiment (Pietikainen et al., 2006), it was demonstrated that fungal and bacterial growth rates had optimum temperatures around 25-30^{o}C, while at higher temperatures lower values were recorded. This decrease they noted was more drastic for fungal than the bacterial community. Pietikainen et al. (2006), further observed that a tendency towards the opposite effect was observed at low temperatures, which seemed to suggest that fungi were more adapted to low-temperature conditions than bacteria; In another investigation of *Steinernema feltiae* (nematode) in soil floors of turkey houses, Geden and Axtell (1988) reported that these nematodes did not survive beyond 2 weeks after treatment in soil held at temperatures > 24^{o}C. However, they observed that these nematodes were still detected at 9 week after treatment in soil at 20 and 24^{o}C; In a study that investigated the impact of soil temperature on the virulence of the entomopathogenic nematodes *Steinernema carpocapsae* and *S. feltiae*, Radova and Trnkova (2010), reported that S. carpocapsae was generally significantly more efficient at the highest temperature (25^{o}C) than *S. feltiae*, while *S. feltiae* recorded higher insect mortality at lower temperatures (15^{o}C and 10^{o}C); In their study of nematode community structure, Bakonyi et al. (2007) noted that the diversity of nematode genera responded to warming regardless of the microhabitat type. Their nematode diversity profile analysis showed that warmed plots had significantly higher diversity of high density species than the control. It is thus clear that different soil microbial communities and sometimes members of same taxa in a soil microbial community respond differently to differing degrees of temperature; In a laboratory experiment it was demonstrated that soil microbial biomass C was positively correlated with soil temperature, while urease activity decreased gradually with temperature and time (Joa et al., 2010). Joa et al. concluded that soil microbial activity showed differences in sensitivity with soil type and temperature.

Objective: An understanding of the relationship between soil nematode groups and temperature could be useful for management purposes and /or environmental changes. This investigation attempts to elucidate how different levels of temperature affect different trophic groups of soil nematode populations, in a natural ecological setting.

Free-living soil nematodes have a major role in ecologic soil processes such as decomposition, mineralization, nutrient cycling, and the suppression of pest species. Therefore any factor e.g temperature, pH, amendments, etc that alters the nematode community composition, may be expected to considerably affect the functioning of the ecosystem.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling

This investigation was carried out in Marche, Pulaski County, Central Arkansas, USA. The soils found here include shale and sandstone, which are sedimentary soils that contain mostly clay and tiny particles of crystals such as quartz (SiO_2). The soils are also described as Silt Loam (Brady & Weil, 2008).

The farm sampled was a mid-sized non-commercial farm. Turnips were grown in tilled plots and the sampled

farm had seven subplots, each approximately 7.5 m x 1.6 m. Each subplot was separated by an approximate 1m gap. Half of the sampled farm was surrounded by a pine forest, one-quarter by a yard lawn and the other one-quarter by other crops. The piece of land on which the turnips were grown has been farmed continuously for at least the last 10 years with different crops in rotation. The last crops to be grown on these plots before the turnips were celery and carrots.

Sampling was done every 4-week from four subplots randomly chosen. Samples were collected from each subplot randomly and in a zigzag manner, using a foot driven soil sampler. Soil samples were collected within a 15-20cm depth and the sampler had a diameter of approximately 1.5 cm. A total of six corer soil samples constituted one composite sample from each subplot randomly determined. Thus for each sampling period, four composite samples were collected, representing four replicates.

Samples were collected for five months, December through April and this included periods of turnip growth and periods of absence of these plants. The soil is normally tilled and limed before planting and this is done in February or March.

2.2 Soil Temperature

A total of five temperature probes were buried in five randomly determined locations in the turnips farm. The probes were of simple design, a pointed end to which was mounted a dial with a temperature scale. The probes were buried into the soil to a depth of 20cm and each week at mid-day the temperature reading taken. There were thus three temperature readings taken before the sampling week, plus the temperature reading during sampling. The mean temperature reading for each month was recorded.

2.3 Nematode Extractions and pH Determination

In the laboratory each composite sample was homogenized, and then a 100 mL subsample was used to extract nematodes, while a 50 mL subsample was used for pH determination. Nematodes were extracted using a combination of decanting, sieving, and Baermann funnels, as essentially descwribed by Barker (1985). A 100 mL soil sample was transferred onto a container with 10 L of water, the clumps were dissolved and the filtrate decanted through 60 um and then 400 um mesh sieves (Matute et al, 2012). The retained sample was then transferred onto a Baermann funnel assemblage and incubated for 72 hours. 15 mL of each sample was tapped and subjected to quantitative and qualitative analysis (Ferris & Matute, 2003). For pH, 50 mL soil sample was dissolved in 100 mL distilled water and the mixture stirred. The stirred mixture was allowed to settle for 40-60 seconds and then the supernatant was decanted onto a beaker. Using a pH meter with an electrode, the pH value of the solution was recorded.

2.4 Data Analysis

A total of three analyses were done: a) Analysis of Variance-was used to determine if populations of nematodes significantly varied during the different sampling periods; b) Correlation coefficients (r) was used to determine if there existed any relationship between pH, temperature and nematode populations; and c) Biomass calculation (Matute et al. 2009, 2013) was used to determine the specific living tissue contributed by each trophic group and members within a trophic group.

For the ANOVA and correlation coefficient (r) analyses, the most abundant subgroup of nematodes in each main ecological functional category was used for the analysis. This was determined by their relative and respective biomass contributions as calculated and presented in each of the tables representing an ecological functional group.

3. RESULTS

3.1 Nitrogen Releasing Nematodes/NRN: Bacterivorous and Fungivorous Nematodes

Four colonizer-persister (cp) groups of nitrogen releasing nematodes (NRN) were recovered (Table 1). They were the bacterial feeding nematodes (Ba) of the cp classes Ba_1, Ba_2, Ba_3, and the fungal feeding nematodes (Fu) of the cp class Fu_2. Over 67% of the NRN were of the Ba_2 group. In decreasing abundance the Ba_2 nematode taxa were *Plectus* (70.23%), *Acrobeloides* (23.72%) and *Cephalobus*(6.05%). The monthly variation of Ba_2 nematode population during the sampling period was significant ($P < 0.01$). While February recorded the highest population of nematodes, April recorded the least (Table 1). The population of bacterivorous nematodes were weakly negatively correlated with pH($r = -0.082$) and mildly negatively correlated with temperature($r = -0.503$).

Table 1
Nitrogen Releasing Nematodes: Mean Number and Biomass of Bacterivorous (Ba) **and Fungivorous** (Fu) **Nematodes per 100 mL soil. Values Are Means of 10 Samples per Sampling Period**

	Month	Dec.	Jan.	Feb.	Mar.	Apr.		
	Temp. °C	5.67	1.32	8.41	12.95	22.43		
	pH	5.51	5.99	5.01	6.93	4.73		
Cp Class	Nema Taxa						Biomass	% Biomass
$Cp1/Ba_1$	*Mesorhabditis*	211.1	110.1	663.8	38.4	0	1,397.3	13.82
	Rhabditis	0	0	0	337.8	13.4		
	Panagrolaimus	0	0	0	0	22.7		

To be continued

Continued

	Month	Dec.	Jan.	Feb.	Mar.	Apr.		
$Cp2/Ba_2$	*Cephalobus*	48.8	46.2	58.6	61.6	0	6, 808.8	67.36
	Acrobeloides	184	281.8	219.5	114.5	5.5		
	Plectus	360	314.4	1, 295.1	372.7	41.7		
$Cp3/Ba_3$	*Prismatolaimus*	11.7	46.3	187.2	52.3	1.6	897.3	8.88
$Cp2/Fu_2$	*Aphelenchoides*	15.7	24.1	221.7	51.1	40.2	1, 005.4	9.95
	Aphelenchus	37.9	17.7	60.2	31.4	2.7		
Biomass		1,539	1, 617.4	4, 935.6	1, 795.7	221.1	10, 108.8	
% Biomass		15.22	16	48.82	17.76	2.19		

Temperatures are means of four readings, one per week and pH are means from the four composite monthly samples.

3.2 Plant-Parasitic Nematodes/PPN: Plant Tissue Herbivores

Three cp classes of plant-parasitic nematodes (Pl) were recorded. They were the Pl_2, Pl_3 and Pl_5, respectively. Parasitic nematodes of the Pl_3 dominated with over 65% of the biomass contributed by these nematodes (Table 2). The most abundant of the Pl_3 nematodes were *Helicotylenchus mucronatus* (74.26%) and *Rotylenchus buxophilus* (21.35%), and then *Meloidogyne* and *Hoplolaimus*. Populations of Pl_3 nematodes varied significantly from month to month during the sampling period ($P < 0.01$). February recorded the highest populations versus the lowest populations in January and April. Populations of the Pl_3 nematodes were weakly negatively correlated with pH($r = -0.32$) and also weakly negatively correlated with temperature($r = -0.199$).

Table 2
Plant-Parasitic Nematodes: Mean Number and Biomass(ug) **of Root Feeding** (Pl) **Nematodes per 100 mL Soil. Values are Means of 10 Samples per Sampling Period**

	Month	Dec.	Jan.	Feb.	Mar.	Apr.		
	Temp. ^oC	5.67	1.32	8.41	12.95	22.43		
	pH	5.51	5.99	5.01	6.93	4.73		
Cp Class	Nema Taxa						Biomass	% Biomass
$Cp2/Pl_2$	Tylenchus costatus	21	34	367.2	55.1	96.9	1, 794.8	16.57
	Psilenchus hilarulus	0	0	312.3	0	10.9		
$Cp3/Pl_3$	Helicotylenchus mucronatus	381.9	55	997.8	216.1	96.2	7, 058.1	65.16
	Meloidogyne microcephala	26.1	49.5	0	0	0		
	Hoplolaimus galeatus	0	27.7	0	0	0		
	Rotylenchus buxophilus	0	0	439.8	62.6	0		
$Cp5/Pl_5$	Xiphinema sp.	102.6	108.1	72.9	60.9	51.3	1, 979	18.27
Biomass		1,779	1, 005.1	6, 036.3	1, 250.8	760.7	10, 831.90	
% Biomass		16.42	9.28	55.73	11.55	7.02		

Temperatures are means of four readings, one per week and pH are means from the four composite monthly samples.

3.3 Pest Suppressor Nematodes/PSN: Carnivorous and Omnivorous Nematodes

Three cp classes of PSN were recovered, they included two cp classes of carnivorous or predatory nematodes (Ca_4 and Ca_5), and one cp class of omnivorous nematodes (Om_4) (Table 3). The omnivores dominated the PSN trophic group constituting 63.89% of the total biomass contributed by this group of nematodes. Among the omnivores *Mesodorylaimus* dominated (61.92%), followed by *Dorylaimus* (38.08%). The fluctuation of populations of Om_4 nematodes during the sampling period was significant ($P < 0.01$). The warmer months (March and April) recorded the lowest populations of these nematodes as compared to the colder months (December-February), in which higher populations of the Om_4 nematodes were recovered. While pH had a weak negative effect on the population of Om_4 nematodes($r = -0.185$), temperature was moderately negatively correlated($r = -0.745$) with the omnivorous nematode populations. The r-value was however statistically not significant.

Table 3
Pest Suppressor Nematodes: Mean Number and Biomass(ug) **of Carnivorous/Predatory** (Ca) **and Omnivorous** (Om) **Nematodes per 100 mL Soil. Values are Means of 10 Samples per Sampling Period**

	Month	Dec.	Jan.	Feb.	Mar.	Apr.		
	Temp. ^oC	5.67	1.32	8.41	12.95	22.43		
	pH	5.51	5.99	5.01	6.93	4.73		
Cp Class	Nema Taxa						Biomass	% Biomass

To be continued

Continued

	Month	Dec.	Jan.	Feb.	Mar.	Apr.		
$Cp4/Ca_4$	*Iontochus*	42.9	0	0	0	0	1, 821.2	35.53
	Mylonchulus	45.6	34.2	210.9	50.1	11.3		
	Mononchus	0	0	0	44.6	15.7		
$Cp5/Ca_5$	*Nygellus*	0	0	0	0	5.9	29.5	0.58
$Cp4/Om_4$	*Mesodorylaimus*	233.4	186.8	0	74.4	12.3	3, 274.8	63.89
	Dorylaimus	0	0	302.6	0	9.2		
Biomass		1, 287.6	884	2, 054	676.4	223.5	5, 225.50	
% Biomass		25.12	17.25	40.07	13.2	4.36		

Temperatures are means of four readings, one per week and pH are means from the four composite monthly samples.

4. DISCUSSION

4.1 Soil Nematode Composition of Brassica Rapa in the Study Area

In the soil food web, there are five main trophic groups of nematodes that constitute the nematode community structure. These nematodes are bacterial feeders (Ba), fungal feeders (Fu), Plant-parasitic nematodes or plant tissue feeders (Pl), Carnivorous or Predatory nematodes (Ca), and the Omnivorous nematodes (Om_4). All of these nematode trophic groups were recovered from the *B. rapa* plots sampled (Tables 1-3). While previous nematological investigations of *B.rapa* had documented the plant-parasitic nematodes associated with this crop, this investigation has documented the beneficial free-living nematodes associated with the soils of *B. rapa*, in addition to the plant tissue feeding nematodes. For example we know now that the bacterivorous nematodes contribute more to N-mineralization in turnip plots than the fungal feeders and also that among the bacterial feeders, the Ba_2 nematodes (mostly *Plectus* and *Acrobeloides*), contribute most to soil fertility in *B. rapa* plots (Ferris & Matute, 2003). The monthly fluctuation of populations for all three main ecologically functional nematode groups followed the same pattern. Their populations peaked and dropped at same times. These seem to suggest an interrelationship between these nematode groups as a web. When the population of plant-parasitic nematode increases, the predator population also increases and an increase in predator population will lead to a decrease in the prey population. Similarly an increase in the population of the NRN will lead to an increase in N-mineralization, which in turn could cushion the effects of parasitic nematodes feeding on root tissues.

Approximately 1000 *H. mucronatus* nematodes/100 mL of soil was recorded. This is 10x the value for which parasitic nematode management is recommended. It is thus concluded that plant-parasitic nematodes are a potential constraint to the profitable production of *B. rapa* in the study area, requiring attention. Previously the plant-parasitic nematode genera listed as frequently encountered with *B. rapa* were *Pratylenchus, Helicotylenchus, Heterodera, Tylenchorynchus, Ditylenchus, Meloidogyne*, and *Paratylenchus* (Mennan & Handoo, 2006; Holgado et al., 2008; Knight et al., 1997). In this investigation seven plant-parasitic nematode genera were recovered (Table 2), they include two genera previously reported- *Helicotylenchus* and *Meloidogyne* and five additions-*Tylenchus, Psilenchus, Hoplolaimus, Rotylenchus*, and *Xiphinema.*

4.2 Temperature and Soil Nematodes

Temperature affected all three main groups of nematodes (NRN, PPN, and PSN) similarly. All of these nematode functional groups were negatively correlated with temperature. While correlation with the NRN and PPN nematodes was weak, temperature correlation with the Om_4 nematodes (PSN) was moderate($r = -0.745$). Though the r-value is not statistically significant, it is high enough to suggest that Om_4 nematodes have a higher level of sensitivity to temperature changes than the other groups of nematodes. Data from this investigation seem to suggest that temperatures between 8-9°C are conducive for the proliferation of all three main nematode functional groups and temperatures outside this range, result in dwindling nematode populations. These results suggest that the nematodes encountered in this investigation are thermophobic.

It is not known why there was a negative correlation with temperature for all the nematode populations and groups, when a positive correlation seems to be the norm in previous studies involving soil microbial communities, including nematodes (Pietikainen et al., 2006; Geden & Axtell, 1988; Radova & Trnkova, 2010; Bakonyi et al., 2007; Joa et al., 2010). An increase in soil temperature stimulates microbial activity. An increase in the bacterial and fungal soil populations with increased soil temperature is expected to result in a surge of the bacterivorous and fungivorous nematodes. Also an increase in soil temperature should lead to an improved plant root system, providing an increased food supply for plant-parasitic nematodes. Invariably an increase in soil temperature should lead to an increase in the predator nematode population. It is concluded that there are other soil factors that are at play here and also that a longer field study period is required, to go beyond five months. It is also concluded that all groups of soil nematodes are similarly sensitive to changes in soil temperatures making them good bioindicators of soil conditions and changes.

4.3 Soil Nematodes and pH

Soil pH affected all three main nematode functional groups (NRN, PPN, and PSN) identically. These ecologically functional groups of nematodes were all negatively correlated with pH. This correlation was weak and slightly higher for the PPN (r = -0.32) as compared to the other nematode feeding groups. All nematode populations peaked in February when the pH was 5.01 and then the nematode populations plunged in March and April, when the pH was respectively 6.93 and 4.73. It is thus clear that acidic conditions below pH5 and pH 6 and above are detrimental to the survival of all groups of nematodes in this study. It will thus seem that pH 5-6 is the ideal in the study area, making these nematodes acidophilic.

In an earlier study Korthals et al. (1996) reported that low pH significantly reduced the number of bacterial-feeding nematodes, whereas the number of hyphal-feeding (fungal-feeding) nematodes increased. In this investigation, low pH significantly reduced the numbers of both bacterial- and fungal-feeding nematodes. Raty and Huhta (2003) reported that limed soils in a mesocosm experiment supported significantly more nematodes that unlimed soils. Also Warner (2009) demonstrated that soybean cyst nematode population densities were highest in soils with pH > 7.0 as compared to soils with pH 5.9-6.5. In this investigation March recorded the highest pH of 6.93. This increase in soil pH is attributed to liming in preparation for planting and unlike studies by Warner (2009) and Raty and Huhta (2003), higher pH values resulted in a reductive nematode effect in this investigation. This difference is attributed to the crop studied and / or local physico-chemical factors of the respective soils studied. *Pratylenchus, Hoplolaimus*, Psilenchinae and Dorylaimodea, thrive best at pH6, while the non-stylet bearing nematodes were recovered most from pH 8.0 (Burns, 1971). The pH ranges reported by Burns to be optimal for nematode recovery are those recorded in this investigation to provide a hostile environment for nematode proliferation. Again while Burns (1971) reports that acidic conditions below pH4 are those detrimental to nematode proliferation, this investigation records an acidic environment below pH5 to be inhospitable to nematode habitation (Matute et al., 2012).

CONCLUSION

All nematode groups recovered responded similarly to changes in their environments. They tend to be mildly acidophilic, are thermophobic, and their abundance is significantly influenced by sampling time and other soil factors. Bacterial feeding nematodes (Ba_2) contribute most to nitrogen mineralization, the omnivorous nematodes (Om_4) are the most abundant and therefore most efficient predators (partly because of their sizes and also that they feed on both plant and animal tissue), and plant-parasitic nematodes (Pl_3) are a potential threat to profitable *B. rapa* production in the study area.

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