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Root-lesion nematodes (*Pratylenchus* spp.) in ornamental plant nurseries – influence of soil texture, acidity, salinity and organic matter content

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ABSTRACT

Root-lesion nematodes are common in ornamental plant nurseries of numerous species. It is thus important to study whether this groups of nematodes depend on the physico-chemical properties of the soil assigned for cultivation of ornamental plants. The aim of this research was to determine relation between *Pratylenchus* spp. and soil conditions in ornamental plant nurseries (with conifers, deciduous trees, and shrubs fields) of in Poland. From this study it follows that the occurrence of root-knot nematodes in soils sampled from plant nurseries was associated with physical and chemical properties of the soil. Nematodes of the genus *Pratylenchus*, especially *P. penetrans*, *P. crenatus*, *P. thornei* and *P. flakkensis*, occurred in low salinity and acidic soils. Root-lesion nematodes also preferred soils with low organic matter contents. *Pratylenchus penetrans* and *P. fallax* preferred sandy soils with low contents of silt and clay. In nurseries of ornamental trees and shrubs, most (70%) of the *Pratylenchus* specimens were collected from loamy sand soils. Occurrence of *Pratylenchus* spp. depended on soil pH and texture. *Pratylenchus penetrans* mainly occurred in sandy and very acidic soils where deciduous plants were cultivated whereas *P. neglectus* and *P. crenatus* were found in all kinds of studied soils.

Key Words: conifers; deciduous plants; *Pratylenchus* spp.; soil properties.

INTRODUCTION

Association of soil nematodes with ornamental trees and shrubs in nursery production is poorly recognized, especially in Poland, where ornamental plant nurseries were developed in the 90's. The economically important group of parasites are endoparasitic nematodes out of which the most important belong to the *Pratylenchus* genus. Among of the root-lesion nematodes (RLN), *Pratylenchus penetrans* in particular is harmful to orchards (Hoestra and

Oostenbrink 1962, Zepp and Szczygiał 1986), forest nurseries (Sutherland 1967) and ornamental plants nurseries (Ohkawa and Saigusa 1981).

Damages caused by RLN depend on their density in the soil and the possibility of their reproduction and feeding on roots under specific soil conditions. Type of soil also affects both the nematode species composition and growth of their host plants. One of the most important physio-chemical factors of the soil is soil acidity, which determines physical, chemical and biological properties of the soil, thereby influencing plant growth and development of soil organism populations. Another important chemical property of the soil is soil salinity and salt accumulation is affected by weather conditions, inadequate watering and plant fertilization. Another basic soil component is organic matter, which is a source of carbon and energy for plants and soil organisms, but also influences root lesion nematodes. The physical and physio-chemical properties of the soil determine also quantitative share of clay, silt and sand present in it (Kabata-Pendias 2000).

The aim of this research was to determine the relation between *Pratylenchus* spp. and soil conditions in ornamental plant nurseries.

MATERIALS AND METHODS

SOIL SAMPLING, NEMATODE EXTRACTION AND IDENTIFICATION

The research was conducted in 2007-2008 in 21 ornamental nurseries in Poland in 20 locations (Figure 1). In order to pinpoint the distribution of nurseries, we used the UTM code with 10/10 km² (Table 1). A total of 114 samples were collected from fields of conifers and 155 samples from fields of deciduous trees and shrubs. Physio-chemical analysis of the soil were made for 168 samples, randomly selected from these 269 samples taken from nurseries (Table 1).

Table 1. Soil properties of sampled soils.

Soil texture	No. of samples	pH mean (range)	Salinity mean (range) [dS/m]	Organic matter mean (range) [%]	Location (in UTM code)
Coniferous nurseries					
sand	23	-	-	-	CA62, CB63, CC27, CC28
loamy sand	8	5.75 (5.4-6.1)	0.125 (0.07-0.18)	5.78 (1.97-3.81)	CD30, DD75, DF22, XS74
sandy loam	19	5.80 (5.0-6.9)	0.14 (0.07-0.22)	3.50 (2.35-4.97)	CA62, CB74, DC94, DD75, CC03, DF22, DF22, CA41, CA53, XS73
sandy clay loam	2	-	-	-	CA41, XS73
silt	10	-	-	-	CA41, CC03, CA62
Deciduous nurseries					
sand	14	5.85 (5.3-6.4)	0.12 (0.09-0.16)	2.45 (1.49-3.42)	CD30, DC96, CA62
loamy sand	25	5.06 (3.7-6.4)	0.08 (0.04-0.17)	1.76 (1.44-1.97)	CD30, XT49, DD75, DF22
sandy loam	51	6.12 (5.0-7.1)	0.18 (0.1-0.26)	3.09 (2.35-3.96)	CA62, DC88, DC94, XS73, CA41, CA53, EB79, DF22, CC03, DD75
sandy clay loam	16	-	-	-	CA41, XS73, CC03, CA41, CA62, EB79

Samples were collected once during the growing season using a soil core sampler with a diameter of 20 mm. A single sample was 200 g of soil, which was taken after mixing soil derived from 10 punctures at a distance of about 50 cm from the trunk to a depth of about 20-

30 cm. Nematodes were washed from fresh weight of soil using an Oostenbrink elutriator (MEKU Erich Pollähne GmbH, Hannover, Germany), the centrifugal machine, and the Baermann method (Hooper 1970). Nematodes for microscopic examination were fixed in 4% formalin solution, and permanent slides were made using the rapid lactoglycerol method (Franklin and Goodey 1949). Nematodes were identified using morphometric measurements using a compound microscope Nikon Eclipse 80i with differential interference contrast (DIC - Nomarski technique) at a power of up to 1000x magnification.

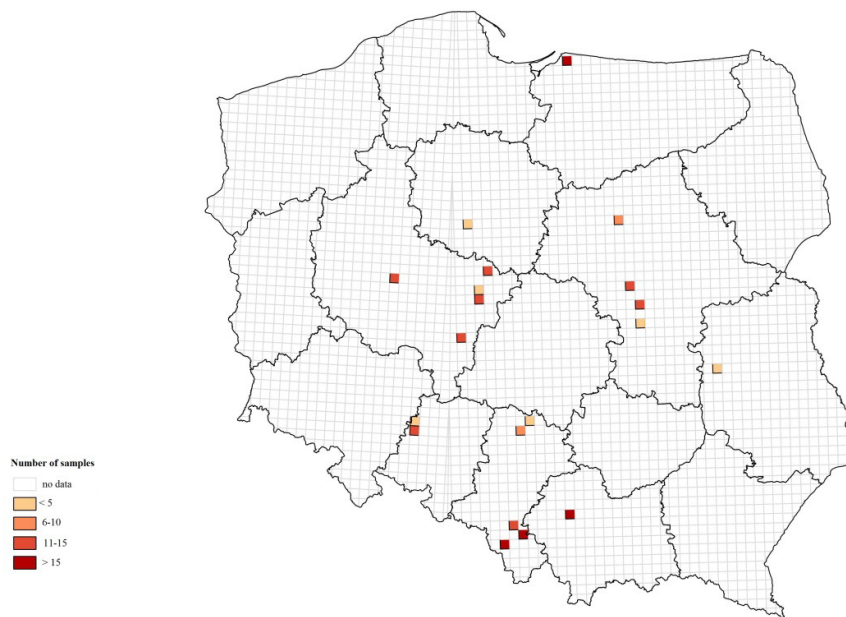


Figure 1. Location of sampling sites

SOIL PARAMETERS

A mixed sample was examined for the following physio-chemical parameters: particle size distribution by Bouyoucosa-Casagrande aerometric method in Prószyński's modification (PN-R-04032); pH in H₂O by electrometric method (PN-ISO 10390); and conductivity by conductometric method (PN-ISO 11265). The content of organic matter was determined from ash formed during incineration for 4 hours at 450 °C (Ostrowska et al. 1991). To evaluate the acidity of the soil the following scale was used (Starck, 1997): highly acidic soil - pH < 4.5; acidic soil value - pH 4.6-5.5; slightly acidic soil - pH 5.6-6.5; neutral soil - pH 6.6-7.2; alkaline soils - pH > 7.2.

Based on content of fractions, soils were classified into granulometric groups and subgroups (Mercik 2002). Agronomic categories of soil were also established. Very light and light soils are sand and loamy sand. Medium and heavy soils are sandy loam, sandy clay loam, loam, clay loam, silt loam, silty clay loam, silt and clay (Grzebisz et al. 2005). The full soil analysis (the content of organic matter, salinity and pH) was performed for loamy sand and sandy loam, as the most often recorded in the nurseries.

Interaction between the number of nematodes and physio-chemical characteristics of the soil was analyzed with Spearman rank correlation. Correlation does not determine a direct causal relationship, but it demonstrates a dependence between the occurrence of nematode and environmental conditions, which was the essence of the study. This method was also used to analyze interaction between the number of nematodes and every species of conifers and deciduous trees and shrubs, but this dependence was observed only in the nurseries

with *Robinia pseudoacacia*. Data were analyzed using Excel Statistic 2007 for Windows® (SSRI) StatSoft, Inc. (2011) and STATISTICA, version 10. (www.statsoft.com). Absolute frequency of occurrence and absolute population density (Norton, 1978) of RLN were calculated per 100 g of soil.

RESULTS AND DISCUSSION

SOIL TEXTURE

More than 70% of RLN all (954 specimens) were observed in loamy sand soil, whereas in sandy loam soils less than 20% specimens were found (Table 2). In silty soils RLN were not found. The loamy sand was the only soil texture where each nematode species was recorded, including *Pratylenchus penetrans* specimens in large numbers. This nematode was also recorded in sandy loam and sandy clay loam, but *Pratylenchus neglectus* was more numerous there (Table 3).

Table 2. Number of *Pratylenchus* spp. in soil found in ornamental plant nurseries, and grain size composition and soil pH.

Soil Texture	RLN		Absolute frequency [%]*	Soil pH	RLN		Absolute frequency [%]
	No.	%			No.	%	
Sand	112	8.3	7.5	Very acid soil (< 4.5)	860	69.9	78.2
Loamy sand	954	70.6	28.4	Acid soil (4.6-5.5)	192	15.6	9.1
Sandy loam	253	18.7	8.7	Light acid soil (5.6-6.5)	102	8.3	3.8
Sandy clay loam	32	2.4	0.6	Neutral soil (6.6-7.2)	77	6.2	4.5
Total	1351	100		Total	1231	100	

* Absolute frequency=number of samples containing a species x100/number of samples collected

Table 3. Distribution of RLN in different soil texture

Species	Total no. of positive samples	Absolute density* [count/100g]				Absolute frequency [%]			
		Sand	Loamy sand	Sandy loam	Sandy clay loam	Sand	Loamy sand	Sandy loam	Sandy clay loam
<i>P. crenatus</i>	15	3.5	7.3	3.0	1.0	5.4	12.1	11.4	6.7
<i>P. fallax</i>	16	19.0	14.5	2.7	1.0	13.5	9.1	10.0	6.7
<i>P. flakkensis</i>	3	0.0	107.0	5.0	0.0	0.0	6.1	1.4	0.0
<i>P. neglectus</i>	14	8.0	4.7	18.8	8.0	5.4	12.1	8.6	13.3
<i>P. penetrans</i>	7	0.0	138.3	2.0	3.0	0.0	12.1	2.8	6.7
<i>P. pratensis</i>	3	0.0	4.0	2.5	0.0	0.0	3.0	2.8	0.0
<i>P. pseudopratensis</i>	2	0.0	10.0	0.0	0.0	0.0	6.1	0.0	0.0
<i>P. thornei</i>	10	4.0	12.5	1.6	7.0	5.4	6.1	7.1	6.7

* Absolute density=mean number of nematodes in all samples

The analysis of Spearman correlation coefficients indicated that RLN responded to sand content in soil. Positive impact of sand content in soil on the number of nematodes inhabiting the soil was observed only for *P. penetrans* occurring in the soil with *Robinia pseudoacacia* (Table 4). There was no significant correlation between soil texture and the presence of other RLN.

Table 4. Spearman r for *Pratylenchus* species and selected soil properties

Nematode species	Plants	Parameter estimates			
		Sand (%)	Soil pH	Soil EC	Organic matter (%)
<i>Pratylenchus penetrans</i>	Deciduous**	0.049	-0.209	-0.288*	-0.061
	<i>Robinia pseudoacacia</i> **	0.664*	-0.801*	-0.801*	-0.801*
<i>P. crenatus</i>	Deciduous	0.052	-0.198	-0.289*	-0.235
	Coniferous**	-0.050	-0.442	-0.458	-0.538*

* correlation statistically significant at $p < 0,05$

** for analyses 89 sample of soil from coniferous nurseries, 132 sample from deciduous nurseries and 10 samples taken under *Robinia pseudoacacia* plants were used.

The significant influence of soil texture on the occurrence of some *Pratylenchus* species has been confirmed by other researchers (Brown et al. 1980, Griffin 1996). A particularly important factor is the sand content (Talwana et al. 2008, Chen et al. 2012). More frequent occurrences of RLN, especially *P. penetrans*, were observed in loamy sand, and were previously observed less frequently in heavy soils (Oostenbrink 1961, Szczygieł and Zepp 2004). According to Szczygieł et al. (1983) populations of this nematode are higher in light soils (0 and 7.5% of clay) than in heavy soils (45 and 60% of clay). Our data indicated the presence of *Pratylenchus* nematodes in sandy clay loam. This is consistent with the observations by Szczygieł and Zepp (2004). They found that *P. crenatus* was most common and most numerous in loam and clay soils, including sandy clay loam. *P. neglectus* also occurred in different types of soil, but less frequently in heavy soil and not in large numbers (Szczygieł and Zepp 2004), although Brzeski (1969) often reported the presence of this nematode in clay and loamy soils.

SOIL ACIDITY

Nematodes of the genus *Pratylenchus* were frequently found in very acidic soils. Only about 15% of specimens (Table 2) were collected in light acidic soils and neutral soils. In both conifer and deciduous tree and shrub nurseries, RLN reacted negatively to a reduction in soil acidity and decreased their number with an increasing soil pH (Table 5). These nematodes were most often found in acidic soils (deciduous plants) and light acidic soils (coniferous plants). A significant negative correlation was found between the acidity of the soil and the occurrence of *Pratylenchus penetrans* in soil with *Robinia pseudoacacia* (Table 4).

Table 5. Distribution of RLN in different soil pH

Species	Total no. of positive samples	Absolute density [szt.100g]				Absolute frequency [%]			
		Very acid soil	Acid soil	Light acid soil	Neutral soil	Very acid soil	Acid soil	Light acid soil	Neutral soil
<i>P. crenatus</i>	10	10.0	3.7	0.0	4.0	18.2	28.6	3.7	5.9
<i>P. fallax</i>	8	0.0	24.7	5.0	1.3	0.0	14.3	7.4	17.6
<i>P. flakkensis</i>	3	212.0	0.0	2.0	5.0	9.1	0.0	3.7	5.9
<i>P. neglectus</i>	7	4.0	36.0	5.0	17.0	9.1	9.5	7.4	11.8
<i>P. penetrans</i>	6	138.3	0.0	0.0	2.0	36.4	0.0	0.0	11.8
<i>P. pratensis</i>	3	0.0	4.0	0.0	2.5	0.0	4.8	0.0	11.8
<i>P. pseudopratenensis</i>	3	6.0	0.0	14.0	0.0	9.1	4.8	3.7	0.0
<i>P. thornei</i>	8	21.0	2.0	4.3	1.0	9.1	4.8	11.1	17.6

The dependency between the presence of *Pratylenchus* nematodes and soil acidity has been ascertained by many authors (e.g., Burns 1971, Willis 1972). The numerous occurrence of *Pratylenchus crenatus* in soils with higher acidity was confirmed by Brzeski (1969), where this nematode was not noticed in alkaline soils, but was often found in very acidic soils (pH below 4.5). *P. crenatus* was also frequent in fruit tree nurseries in acidic soils (pH 5.9) (Szczygieł et al. 1969). The presence of a large number of *P. penetrans* specimens in very acidic soil found in the present study is not consistent with the report by Szczygieł and Zepp (2004), in which this nematode was present in greater numbers in less acidic soils, especially at pH > 6.5.

SOIL SALINITY

A significant negative correlation between soil salinity and the occurrence of *P. penetrans* and *P. crenatus* was noted in the deciduous plants nurseries. A particularly strong correlation was noted between this factor and the number of *P. penetrans* in the *R. pseudoacacia* fields (Table 4). The negative correlation between the number of nematodes and soil salinity is in accordance with previous observations (Skwierz 1989).

ORGANIC MATTER CONTENT

Higher content of soil organic matter in conifer nurseries resulted in lower number of *Pratylenchus* nematodes, especially *P. crenatus*, and *P. penetrans* in *R. pseudoacacia* nurseries (Table 4). The negative correlation between the number of most *Pratylenchus* species and the content of organic matter in the soil confirms results by other authors (e.g., Muller and Gooch 1982, Szczygieł and Zepp 1983).

In summary, the results obtained indicate that edaphic factors, such as soil acidity, soil texture, and organic matter content, affect the number of root-knot nematodes. Thus, suitable soil conditions and appropriate host plants may favour nematode development to an extent that may affect plant production in ornamental nurseries.

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