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NEMATODES IN THE VINEYARDS IN THE NORTHWESTERN PART OF POLAND

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Abstract. The knowledge on nematodes occurrence in Polish vineyards is poor. The surveys of the species from the rhizosphere of plants were conducted between 2013 and 2014 in 12 vineyards in the northwestern part of Poland. Recovery of the nematodes was made in two steps. First, through incubation of 50 g of the roots on sieve. Second, by centrifugation method using 200 g of soil. Nematodes obtained were killed by hot 6% formaline and then processed to glycerine. Permanent slides were determined to the species using keys. During this process there were obtained nematode species from which 12 belonged to genus of fungivorous, 4 to genus of bacteriavorous and 38 to plant parasitic species. Ten of them are known as nematode vectors of plant viruses (GYFV, CLRV, TRV, AMV, SLRV, GLRaV-1, -2, -3, GVA, GVB, GVE, GFLV, GCMV, GrSPaV, GFkV, GRSPaV). Nematode fauna of vineyards needs broadly searching, especially nematode vectors of plant viruses, which are serious enemy to the vineyards. Studies on *Aphelenchoides ritzemabosi* in vine plants disease complex are necessary.

Key words: virus vectors, nematode fauna, *Vitis* L.

INTRODUCTION

Accession of Poland to the European Union and inclusion of the entire area of vineyards to the zone A of European viticulture involved an annual increase of vineyard areas since 2005, which now establish over 1000 ha [Komorowska et al. 2014]. The fulfillment of 70% required wine consumption in Poland will be possible at the level of 35.000 ha of vineyard areas during next ten years [Wilk 2013].

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The harmfulness of nematodes to vine plants has been noted by numerous authors [Weischer 1961, Pinkerton et al. 1999, Bello et al. 2004, Teliz et al. 2007, Deimi and Mitkowski 2010] and only one from Poland [Wiśniewska et al. 2013]. In Europe there were found up to 300 species of plant parasitic nematodes which occurred near vine plant's rizosphere [Bello et al. 2004]. In 1997, Digiaro et al. [1997] reported about complex virus diseases on decreasing vine yield by 23% and sugar level by near 5%.

Nematodes can interact with soil inhabiting fungi, bacteria, viruses, viroids and phytoplasmas infecting plants [Skwiercz 1987, Dmowska et al. 2013] being also responsible for replanting diseases in fruit plantations [Hoestra 1994, Szczygieł and Zepp 1998, 2004]. The feeding of nematodes may have multiple effects on vine plants, also in decreasing their frost and drought resistance [Pinkerton et al. 1999]. Nematodes weak their resistance to fungus and bacterial pathogens, directly or indirectly are responsible for replanting disease complexes of vine.

Genus *Paralongidorus*, *Longidorus*, *Xiphinema*, *Paratrichodorus* and *Trichodorus.* The group of the most pest ectoparasite nematodes. During feeding on the roots of virus infected plants, nematode becomes a vector and transfers virus, viroids or phytoplasmas to the roots of healthy plants. Komorowska et al. [2012, 2014] found presence of plant viruses in 82.6% of Polish vineyards. In European vineyards nematode vectors of plant viruses are common in all types of soil [Lamberti et al. 1975], listed on the European Quarantine List, as *Longidorus diadecturus* Eveleigh and Allen 1990 [Karnkowski 2004]. In the first Polish survey of vineyard nematodes [Wiśniewska et al. 2013] only *Xiphinema vuittenezi* [Luc, Lima, Weischer and Flegg 1964] were found. The next survey made by Skwiercz and Dzięgielewska [2014] found 2 species of *Xiphinema*, 3 species of *Longidorus* and 5 species of Trichodoridae. All these species are vectors of plant viruses.

Genus *Pratylenchus* and *Meloidogyne.* Migratory endoparasites enable penetration of cortical tissues of the young roots and lead to their deterioration by bacterial (mostly Agrobacterium) and fungus diseases caused by Fusarium, Phytophtora, Sclerotinia and Verticilium [Skwiercz 1987]. Serious illnesses involve feeding of infecting larval of Meloidogyne species. Feeding inside cortical tissues of the young roots of mature female involve gall and deforming the root system.

Genus *Mesocriconema*, *Criconemoides*, *Helicotylenchus*, *Rotylenchus.* Semi-endoparasites feeding with a part of his body inside roots of the host plants. Most prevalent species are *Mesocriconema xenoplax* [Raski 1952], and two spiral nematodes, *Rotylenchus robustus* [De Man 1876] and *Helicotylenchus digonicus* [Perry 1959].

Polish investigation of vineyard nematofauna is lacking. The aim of the present study was to fill, partly, this gap.

MATERIAL AND METHODS

The investigations were carried out in 2012–2013, in 12 vineyards of the 3 provinces of the northwestern part of Poland: West Pomerania – 18 samples (Baniewice VV 4 3d, Mierzęcin VU 4 2d, Maszewo WV 1 2d, Pomerania) – 4 samples (Głobin XA 3 4b, Dębogórze CF 2 4a) and Wielkopolska – 6 samples (Poznań Ławica XU 4 4c, Suchy

Las XU 4 3b). Universal Transverse Mercator (UTM) – grid map of Poland was used after Kornobis [1980]. Each sample was taken from the depth of 45 cm in the vicinity of vine roots. Recovery of nematodes was made in two steps. Larger nematodes (Longidoridae and endoparasite Pratylenchidae) were extracted from 50 g of the roots by Baermann method (incubation on sieve during 4 days), while smaller species were extracted from 200 g of the soil sample by centrifugation [Szczygieł 1971]. Nematodes obtained from incubation and centrifugation were combined in water and killed by adding similar amount of 6% hot formaline. After processing to glycerine by Seinhorst rapid method [Seinhorst 1962], permanent slides of nematodes were made. Plant parasiting nematodes and Ditylenchus were examined for species indentification using keys of Brzeski [1998] and Loof and Luc [1990]. Bacteria and fungal feeders genera were recognized by the key of Andrássy [2007].

According to Wiśniewska et al. [2013] type of permanent index was used.

Number of samples with species present Na
Number of samples N

$$C = (Na \times N^{-1}) \times 100\%$$

Types of permanent index C, by Tischler scale [Trojan 1981] as follow:
0–25% occasional species,
26–50 % accessory species,
51–75 % permanent species.

RESULTS AND DISCUSSION

Table 1 listed nematode species found in 28 surveyed samples from the vineyards of the northwestern part of Poland with frequency of occurrence index (in %). Systematic classification of nematodes, identified to species level, was based on the filogenetic system accepted in Fauna Europea [Winiszewska 2008]. Table 2 listed nematode vectors of plant viruses and its relative viruses.

I. Plant parasitic nematodes

Total 38 species were found in 28 soil samples collected from surveyed vineyards. There were 8 permanent species, 15 accessory and 15 occasional species.

Nematode vectors of plant viruses

Genus *Xiphinema*. *X. vuittenezi* were more encountered species from the genus. It could be a serious pest of vine plants in Poland. Kumari [2004] and Kumari et al. [2005] found them in 93% of Czech vineyards; were also noted in Germany [Hubschen et al. 2004] and Slovakia [Liskova 1997]. *X. vuittenezi* and *X. diversicaudatum* can transfer serious plant viruses danger to vine plantations (tab. 2). Seven viruses (GLRaV-1, -2, -3, GVA, GFLV, GFkV, GRSPaV) were found in Polish vineyards by Golis and Komorowska [2011] and Komorowska et al. [2012, 2014]. Viruses were found in plants alone and as complexes virus diseases. Furthermore next viruses can damage vine because of its presence (and nematode vectors too) in Polish soils. Possibility of the virus disease are as follow: GYFV, CLRV, TRV, AMV, SLR (see tab. 2). The nematode vectors of that viruses were found in surveyed vineyards (tabs 1, 2). *L. elongatus*, *L. attenuatus* and *L. piceicola* found in our survey were also noted from Slovakian [Liskova 1997] and Czech [Kumari 2004, Kumari et al. 2005] vineyards. The species are also vectors of plant viruses founded by Komorowska et al. [2012, 2014].

Trichodorus primitivus occurs more frequently from all 10 nematode vectors in our study. With 4 other trichodorids, it can transfer TOBRA viruses to the roots of vine plants.

Table 1. List of nematodes collected from the vineyards of North-Western Poland

	Nematode species	Frequency ofoccurrence (%)
Xiphinematidae	*Xiphinema diversicaudatum* [Micoletzky 1927]	25
	X. vuittenezi [Luc et al. 1964]	43
Longidoridae	*Longidorus attenuatus* [Hooper 1961]	7
	L.elongatus [De Man 1876]	14
	L.piceicola [Liskova et al. 1997]	11
Trichodoridae	*Paratrichodorus pachydermus* [Seinhorst 1954]	43
	P. teres [Hooper 1962]	29
	Trichodorus primitivus [De Man 1880]	54
	T. similis [Seinhorst 1963]	36
	T. viruliferus [Hooper 1963]	36
Criconematidea		
Criconemoides	*Criconemoides informis* [Micoletzky 1922]	43
Mesocriconema	*Mesocriconema curvatum* [Raski 1952]	36
	M. rusticum [Micoletzky 1915]	50
	M. solivagum [Andrassy 1962]	29
	M. xenoplax [Raski 1952]	57
Hemicycliophora [De Man 1921]	*Hemicycliophora triangulum* [Loof 1968]	11
Loofia [Siddiqi 1980]	*L. thienemanni* [Schneider 1925]	7
Paratylenchidae	*Paratylenchus bukowinensis* [Micoletzky 1922]	11
	P. projectus [Jenkins 1960]	21
Telotylenchidae		
Bitylenchus [Filipjev 1934]	*B. dubius* [Butschli 1873]	75
Sauertylenchus [Sher 1974]	*S. maximus* [Allen 1955]	21
Merlinius [Siddiqi 1970]	*M. brevidens* [Allen 1955]	43
	M. joctus [Thorne 1949]	14
	M. microdorus [Geraert 1966]	21
	M. nothus [Allen 1955]	14
Scutylenchus [Jairajpuri 1971]	*S. quadrifer* [Andrassy 1954]	43
Tylenchorhynchus [Cobb 1913]	*T. clarus* [Allen 1955]	29
Meloidogynidae	*Meloidogyne hapla* [Chitwood 1949]	25
Hoplolaimidae		
Helicotylenchus [Steiner 1914]	*H. canadensis* [Waseem 1961]	43
	H. digonicus [Perry 1959]	57
	H. pseudorobustus [Steiner 1914]	36
	H. vulgaris [Yuen 1964]	39
Rotylenchus [Filipjev 1936]	*R. pumilus* [Perry 1959]	57
	R. robustus [De Man 1876]	75

Pratylenchidae		
Pratylenchus [Filipjev 1936]	*P. fallax* [Seinhorst 1968]	64
	P. penetrans [Cobb 1917]	54
	P. silvaticus [Brzeski 1968]	25
	P. thornei [Sher and Allen 1953]	25
Nicienie bakteriożerne		
Diplopeltidae	*Cylindrolaimus* [De Man 1880]	14
Rhabditidae	*Rhabditis* sp. [Dujardin 1845]	75
Diplopeltidae	*Cylindrolaimus* [De Man 1880]	14
Plectidae	*Anaplectus* [De Coninck and Schuurmans Stekhoven 1933]	39
	Plectus sp. [Bastian 1865]	50
Nicienie grzybożerne		
Aphelenchoides [Fisher 1894]	*A. angusticaudatus* [Eroshenko 1968]	39
	A. composticola [Franklin 1957]	32
	A. parietinus [Bastian 1865]	32
	A. saprophilus [Franklin 1957]	21
Aphelenchus [Bastian 1865]	*A. avenae* [Bastian 1865]	50
	A. eremitus [Thorne 1861]	21
Ditylenchus [Filipjev 1936]	*D. convallariae* [Sturhan and Friedman 1965]	21
	D. kheiri [Fortunek and Maggenti 1987]	14
	D. medicus [Thorne and Malek 1968]	14
Coslenchus [Siddiqi 1970]		50
Filenchus [Andrassy 1954]		32
Tylenchus [Bastian 1865]		50
Tylencholaimellus [Cobb 1915]		29
Tylencholaimus [De Man 1876]		29

Genus *Paratylenchus*. The sedentary ectoparasites was noted with low frequency of occurrence near vine system roots. Only in Canada they were found in 83.6% of vineyards [Belair et al. 2001].

Genus *Bitylenchus*, *Sauertylenchus*, *Merlinius*, *Scutylenchus*, *Tylenchorhynchus*. From eight species known as migratory ectoparasites, only *B. dubius* was noted frequently. This species occur in all types of soil, on many host plants, but it seems that its harmfulness to vine is low.

Genus *Mesocriconema*, *Hemicycliophora*, *Loofia*. Migratory ectoparasite nematodes occurs commonly in European vineyards (only *M. xenoplax* in some hosts can be a semi-endoparasite) [Wiśniewska et al. 2013, Skwiercz and Dzięgielewska 2014]. They were noted in 70% of German vine plantations [Weischer 1961], but also in Switzerland [Guntzel et al. 1987], in France [Scotto-La Massese et al. 1973] and in Spain [Pinochet and Cisneros 1986]. Our results are also similar in case of *C. informis* and *M. rusticum* to ones from European vineyards [Antoniou 1981, Weischer 1961, Wiśniewska et al. 2013].

Hemicycliophora triangulum and *Loofia thienemanni* are probably occasional species in vine rizosphere, however further observations are required.

Genus *Rotylenchus*, *Helicotylenchus*. Spiral nematodes (semi-endoparasites) during feeding part of body embed into cortical tissues of young roots, but they did not develop inside plant. In our study *R. robustus*, *R. pumilus*, *H. digonicus* are more frequently spiral species, than others species we found. Also in Switzerland [Guntzel et al. 1987] and Spain [Teliz et al. 2007] they were noted often. Due to interaction with other soil pathogens and high frequency of occurrence in vineyards, all spiral nematodes we found could be potentially harmful to vine plants.

Genus *Pratylenchus*. There were four migratory endoparasites from that genus (tab. 1). *P. penetrans* is the most harmful nematode species from all noted in our study. Feeding in the cortical tissues of the young roots leads to necrosis and death of the plant due to phytotoxins, arising during hydrolysis of phenolic substances from enzymes excreted by nematodes. They are harmful, both directly and indirectly, due to the interaction with pathogenic fungi [Skwiercz 1987, Szczygieł and Zepp 1998].

Genus *Meloidogyne*. Specimens of *M. hapla* (larvae) were found in 25% of the surveyed vineyards. This sedentary endoparasites is seriously harmful to many vegetable and fruit plants, also to vine. Infection of larvae develops inside the young roots forming gall, deforming root system. The growth of plant is more affected in dry and poor soils. Root-knot nematode grows more rapidly in the roots infected by viruses then in virus-free roots. [Ravichandra 2014]. Highest population density characterized light soils after growing vegetables. Decreasing population density of root-knot nematode is possible after 2–3 years of growing non-host plants, for example grains.

Table 2. Grapevine viruses detected in Poland and species of nematodes, potentially vectors, occurred in soils of vineyards of North-Western Poland

	Virus	Nematode species – faculty vector of virus
Virus	GLRaV-1 grapevine leafroll associated-virus	*Xiphinema vuittenezi*, *X. diversicaudatum*
	GLRaV-2	*X. vuittenezi*, *X. diversicaudatum*
	GLRaV-3	*X. vuittenezi*, *X. diversicaudatum*
	GVA grapevine virus A	*X. vuittenezi*, *X. diversicaudatum*
	GVB grapevine virus B	*X. vuittenezi*, *X. diversicaudatum*
	GVE grapevine virus E	*X. vuittenezi*, *X. diversicaudatum*
	GFLVgrapevinefanleafvirus	*X. americanum**, *X. diversicaudatum*, *X. index**
	GFKV grapevine fleck virus	*X. americanum**, *X diversicaudatum*, *X. index**
	GRSPaV grapevine rupestris stem pitting	*X. americanum**, *X. diversicaudatum*, *X. index**
Associated virus	GCMV grapevine chrome mosaic virus	*X. americanum**, *X. diversicaudatum*, *X. index**
NEPO viruses	ArMV arabis mosaic virus	*X. diversicaudatum*, *L. elongatus*
	RRV raspberry ringspot virus	*X. diversicaudatum*, *L. elongatus*, *Paralongidorus maximus**
	TomRSV tomato ringspot virus	*X. americanum**
	TBRV tobacco black ring virus	*X. americanum**, *L. attenuatus*
	SLRV strawberry latent ringspot virus	*X. diversicaudatum*, *X. coxi**
	TobRSV tobacco ringspot virus	*X. americanum**, *P. maximus**
TOBRA viruses	TRV tobacco rattle virus	*Paratrichodorus teres*, *P. pachydermus*, *Trichodorus cylindricus**, *T. primitivus* *T. similis*, *T. viruliferus*

**Paralongidorus maximus*, *X. americanum*, *X. coxi*, *X. index* and *T. cylindricus* are common in Polish soils (not found in this survey)

II. Bacteria feeders

Only nematodes from the genus *Rhabditis* occurred frequently (tab. 1). Relatively, populations of bacteria feeders in surveyed vineyards were poor in species and population densities. Soil quality depends on the food web structure, which is largely constructed by nematodes of different trophic groups: bacterial, fungal, plant feeders, omnivores and predators involved to the soil processes like matter circulation and energy flow. It provided stability of the soil system and its resistance to disturbance.

III. Fungal feeders

Four species of Aphelenchoides and two species of Aphelenchus were obtained during the survey of vineyards. *A. ritzemabosi*, migratory foliar feeding nematode cause angular leaf spots, dwarfing, leaf wilt and sometimes forming gall on leaf. It is a parasite to almost 200 species of plants. *A. ritzemabosi* can build specific relation with virus diseased vine plants. The damage by a combination of the virus and nematode pathogens is greater than would be evident when either pathogen is present alone [Ravichandra 2014]. However, *A. avenae* known as hyphal feeder, common and abundant species should affect the functioning of the environment. This effect is positive when *A. avenae* feeds on plant pathogenic fungi – e.g. Fusarium, but negative when nematodes feeds on mycorrhizal fungi. The most diverse and numerous are nematode species belonging to the fungal feeding group followed by epidermal cell feeders and root hair feeders e.g. Coslenchus, Filenchus, Tylenchus, Tylencholaimellus and Tylencholaimus (tab. 1).

CONCLUSIONS

1. Nematode fauna of Polish vineyards needs broadly searching in main areas of the plantations.
2. Nematode vectors of plant viruses (especially latent) are serious enemies to the vineyards.
3. Studies on *A. ritzemabosi* and other foliar feeding nematodes in vine plants disease complex are necessary.
4. Survey of different trophic groups of vineyards nematofauna (omnivorous and predators) should be continued.
5. Bacterivorous nematodes was very poor in species and low population density.

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NICIENIE WINOROŚLI PÓŁNOCNO-ZACHODNIEJ POLSKI

Streszczenie. Występowanie nicieni w uprawie winorośli w Polsce jest słabo opracowane. Badania gatunków nicieni wokół korzeni winorośli przeprowadzono w latach 2013–2014 w 12 winnicach na terenie północno-zachodniej Polski. Nicienie pozyskiwano w 2 etapach: inkubacji z 50 g korzeni na sitach oraz metodą wirówkową z 200 g gleby. Nicienie zabite 6% gorącą formaliną przeniesiono do gliceryny. Stałe preparaty oznaczano do gatunku z użyciem kluczy. Podczas badan pozyskano gatunki nicieni, z których 12 należało do rodzajów nicieni grzybożernych, 4 do rodzaju bakteriożernych i 38 gatunków nicieni pasożytów roślin. Dziesięć z nich uznaje się za wektory wirusów roślinnych. Fauna nicieni winorośli wymaga szerszych badań, zwłaszcza nicieni przenoszących wirusy roślinne, które są poważnym zagrożeniem dla upraw winorośli. Celowe są też badania nad rolą *Aphelenchoides ritzemabosi* w kompleksowej chorobie winorośli.

Słowa kluczowe: wektory wirusów, fauna nicieni, *Vitis* L.

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