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Longidoridae and nepoviruses in Bulgaria and Slovenia

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Summary

Data on the distribution of Longidoridae and nepoviruses in Bulgaria and Slovenia are summarized. Six species of *Longidorus* (*L. apulus*, *L. attenuatus*, *L. arthensis*, *L. fasciatus*, *L. elongatus*, *L. macrosoma*), one *Paralongidorus* species (*P. maximus*) and three *Xiphinema* species (*X. diversicaudatum*, *X. index*, *X. rivesi*) are known as natural vectors of nine nepoviruses in Europe. Currently, 10 and 13 species of *Xiphinema*; 6 and 15 of *Longidorus* are reported to occur in Slovenia and Bulgaria, respectively. *Paralongidorus maximus* has been reported only in Bulgaria. Among the virus vector species *X. index*, *X. diversicaudatum* and *L. elongatus* occur in both countries, *X. rivesi* only in Slovenia and *L. attenuatus*, *L. macrosoma*, *X. italiae* and *P. maximus* only in Bulgaria. A report of *X. index* and Grapevine fanleaf virus (GFLV) in Bulgaria was related to transgenic grape tolerance to the same virus. Nepoviruses have been reported from Slovenia, but despite an evident relationship in the occurrence of GFLV and *X. index* in several vineyards the only laboratory proven transmission is that of TRSV and ToRSV to bait plants by a Slovenian population of *X. rivesi*.

Keywords: distribution; longidorids; *Longidorus*; nepoviruses; *Xiphinema*

Introduction

The discovery of the transmission of grapevine fanleaf nepovirus (GFLV) by *Xiphinema index* Thorne & Allen, 1950 in vineyards in California (Hewitt *et al.*, 1958) and subsequent studies in Europe (Harrison, 1961, 1964; Cadman, 1963) stimulated research on virus vector nematodes and their associated viruses and interactions in different crops (Taylor & Brown, 1997; MacFarlane *et al.*, 2002). Much progress has been achieved in elucidating the systematics, taxonomy, phylogeny, ecology and distribution of the family Longidoridae (Alphey & Taylor, 1986; Hunt,

1993; Coomans *et al.*, 2001; Ye *et al.*, 2004; He *et al.*, 2005) and methods for identifying species, including those of economic importance, have been developed (Abrantes & Santos, 1997; Arias & Bravo, 1997; Brown & Halbrendt, 1997; Ye & Robbins, 2004; De Luca *et al.*, 2004; Hübschen *et al.*, 2004). However, the taxonomic status of most species belonging to the *Xiphinema americanum* group, which are the principal nematode vector species in North America, remains controversial, with many species showing rather uniform morphology and separated only by minor differences (Lamberti *et al.*, 2000). Sampling and extraction methods for virus vector nematodes are reviewed by Brown and Boag (1997) and Ploeg and Brown (1997). A considerable amount of effort has been focused on the transmission process resulting in the development of methods and criteria for assessing the virus transmission of plant viruses by longidorid nematodes (Trudgill *et al.*, 1983) and better understanding of the discrete processes and mechanisms involved in the successful transmission and virus and vector specificity (Brown & Weischer, 1998; MacFarlane *et al.*, 2002).

Particular effort has been devoted to developing techniques enabling detection and identification of nepoviruses both in nematodes and in plants, including sap transmission tests, electron and confocal microscopy, and molecular techniques (Duarte & Brown, 1997; Wang & Gerderich, 1998; Digiaro *et al.*, 2007).

Currently, eight *Longidorus*, one *Paralongidorus* and nine *Xiphinema* species have been proven as natural vectors of 12 of the 38 putative members of the genus *Nepovirus* (MacFarlane *et al.*, 2002).

These viruses threaten crop production by reducing yield, affecting quality, delaying ripening, changing aromatic profiles, and limiting the economic life-span of affected agricultural and horticultural sites. The purpose of this study is to provide a review of the distribution of longidorid nematodes in Bulgaria and Slovenia based on literature sources and new samplings.

Materials and methods

Soil samples were collected about the roots of host plants from different locations in Slovenia and Bulgaria. Sampling was carried out with an auger at 10 – 30 cm depth. Approximately 500 cm³ of a collected soil sample was gently mixed and divided to 200 cm³ sub-samples. Nematodes were extracted from the soil using a decanting method (Hooper, 1986) followed by the Baermann funnel technique. Extracted longidorid nematodes were picked by hand and fixed in TAF or 4% formalin solution for morphometrical identification of the species.

The geographical data of locations were collected from the literature and with GPS device in case of findings after

1999. The nematodes distributions maps were created using ArcGIS 9.3 (ESRI, Redlands, USA) software.

Results and discussion

At present, six species of *Longidorus*: *L. apulus* Roca and Bleve-Zacheo, 1977, *L. arthensis* Brown, Grunder, Hooper, Klinger and Kunz, 1994, *L. attenuatus* Hooper, 1961, *L. elongatus* (De Man, 1876) Thorne and Swanger, 1936, *L. fasciatus* Roca and Lamberti, 1981, *L. macrosoma* Hooper, 1961, one of *Paralongidorus*: *P. maximus* (Bütschli, 1874) Siddiqi, 1964) and three species of *Xiphinema*: *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, *X. index* Thorne and Allen, 1950, *X. rivesi*

Table 1. Longidorid species and their associated viruses reported to occur in Bulgaria (BG) and Slovenia (SLO)

Species	BG	SLO	Viruses	BG	SLO
<i>Longidorus aetnaeus</i>	+				
<i>Longidorus attenuatus</i>	+		Raspberry ringspot (RpRSV) Tomato black ring (TBRV)	+	+
<i>Longidorus distinctus</i>	+				
<i>Longidorus caespiticola</i>	+ ²	+			
<i>Longidorus closelongatus</i>	+				
<i>Longidorus elongatus</i>	+	+	Raspberry ringspot (RpRSV) Tomato black ring (TBRV)	+	+
<i>Longidorus euonymus</i>	+				
<i>Longidorus fagi</i>	+				
<i>Longidorus fasciatus</i>			Artichoke Italian latent (AILV)		+
<i>Longidorus helveticus</i>		+			
<i>Longidorus intermedius</i>	+				
<i>Longidorus leptcephalus</i>	+	+			
<i>Longidorus juvenilis</i>		+	Raspberry bushy dwarf virus (RBDV) ¹		+
<i>Longidorus macrosoma</i>	+		Raspberry ringspot (RpRSV)	+	
<i>Longidorus moesicus</i>	+	+			
<i>Longidorus pisi</i>	+				
<i>Longidorus profundorum</i>	+				
<i>Longidorus vineacola</i>	+				
<i>Paralongidorus maximus</i>	+		Raspberry ringspot (RpRSV)		
<i>Xiphinema basilgoodeyi</i>		+ ²			
<i>Xiphinema diversicaudatum</i>	+	+	Arabis mosaic (ArMV) Strawberry latent ringspot (SLRSV)	+	+
<i>Xiphinema globosum</i>	+				
<i>Xiphinema italiae</i>	+		Grapevine fanleaf (GFLV)	+	
<i>Xiphinema index</i>	+	+	Grapevine fanleaf (GFLV)	+	+
<i>Xiphinema ingens</i>	+ ²				
<i>Xiphinema neovittenezi</i>	+				
<i>Xiphinema pirinense</i>	+				
<i>Xiphinema rotundatum</i>		+ ²			
<i>Xiphinema turicum</i>	+ ²				
<i>Xiphinema vittenezi</i>	+	+			
<i>Xiphinema americanum</i> sl	+				
<i>Xiphinema brevicollum</i>	+				
<i>Xiphinema incertum</i>	+				
<i>Xiphinema rivesi</i>		+	Tobacco ringspot (TRSV) Tomato ringspot (ToRSV)	+	+
<i>Xiphinema simile</i>	+				
<i>Xiphinema pachtaicum</i>	+	+			
<i>Xiphinema parasimile</i>	+				
<i>Xiphinema taylori</i>	+				
Vector unknown			Grapevine Bulgarian latent virus (GBLV)	+	

¹ The role of *L. juvenilis* in RBDV transmission is under investigation (see text).

² should be confirmed with additional investigations

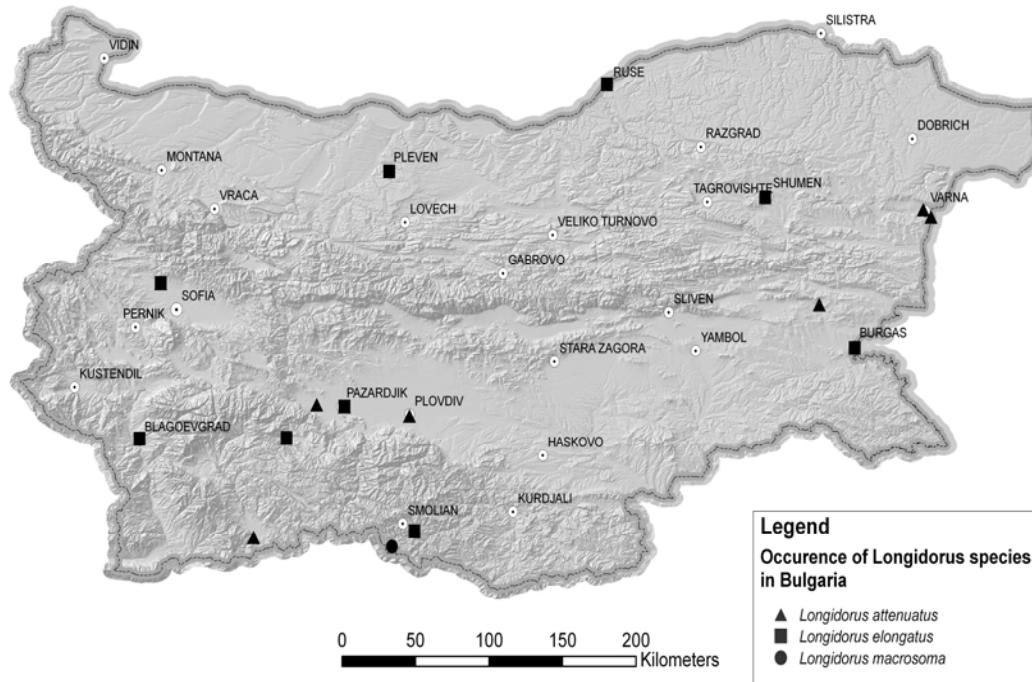


Fig. 1. Distribution of virus vector nematode species of the genus *Longidorus* in Bulgaria

Dalmasso, 1969, are known as natural vectors of nine nepoviruses in Europe (MacFarlane *et al.*, 2002; Širca *et al.*, 2007b.; Mavrič Pleško *et al.*, 2009) also *X. italiae* Meyl, 1953, which has been reputed to transmit GFLV in Israel (Cohn *et al.*, 1970), occurs in Europe. In a series of experiments (Catalano *et al.*, 1992), this virus was not detected in, nor transmitted by *X. italiae* populations originating from vineyards in southern Italy. Recently,

GFLV was detected in *X. italiae* specimens from a vineyard in south-western Bulgaria (Širca *et al.*, 2010). Currently, 10 and 13 species of *Xiphinema*; 6 and 15 of *Longidorus* are reported to occur in Slovenia (Urek *et al.*, 2003a, b; Urek & Širca, 2005; Širca & Urek, 2004; Širca *et al.*, 2007a, b; Širca & Urek, 2009) and Bulgaria (Lamberti *et al.*, 1983; Peneva *et al.*, 2005; Lazarova *et al.*, 2008; Mincheva *et al.*, 2008), respectively (Table 1). However,

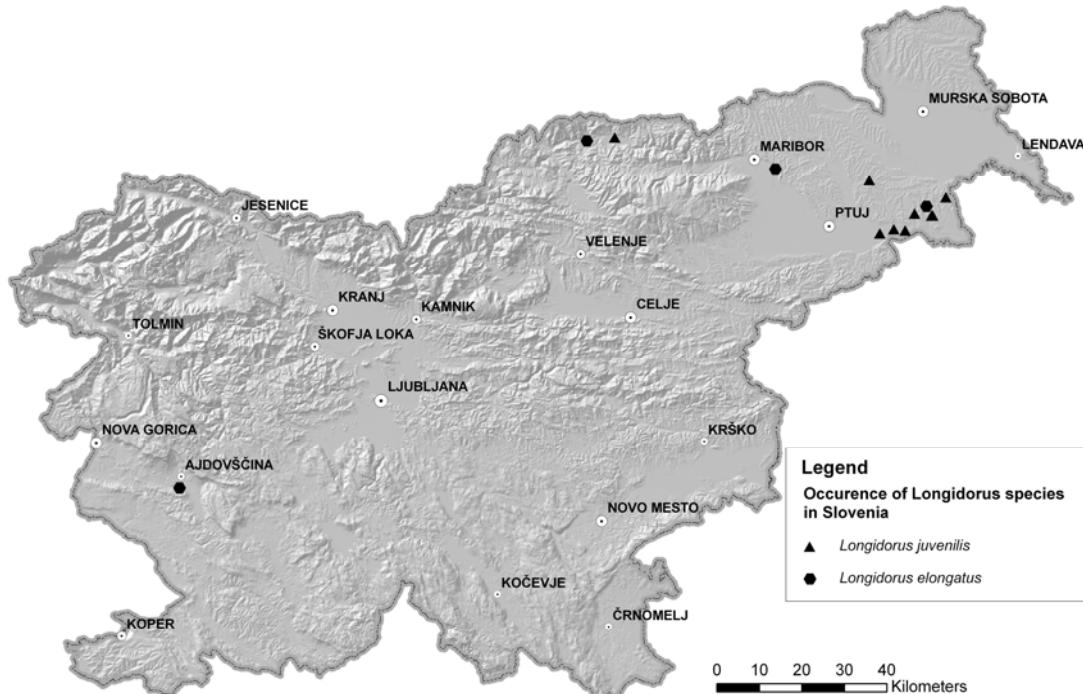


Fig. 2. Distribution of virus vector nematode species of the genus *Longidorus* in Slovenia

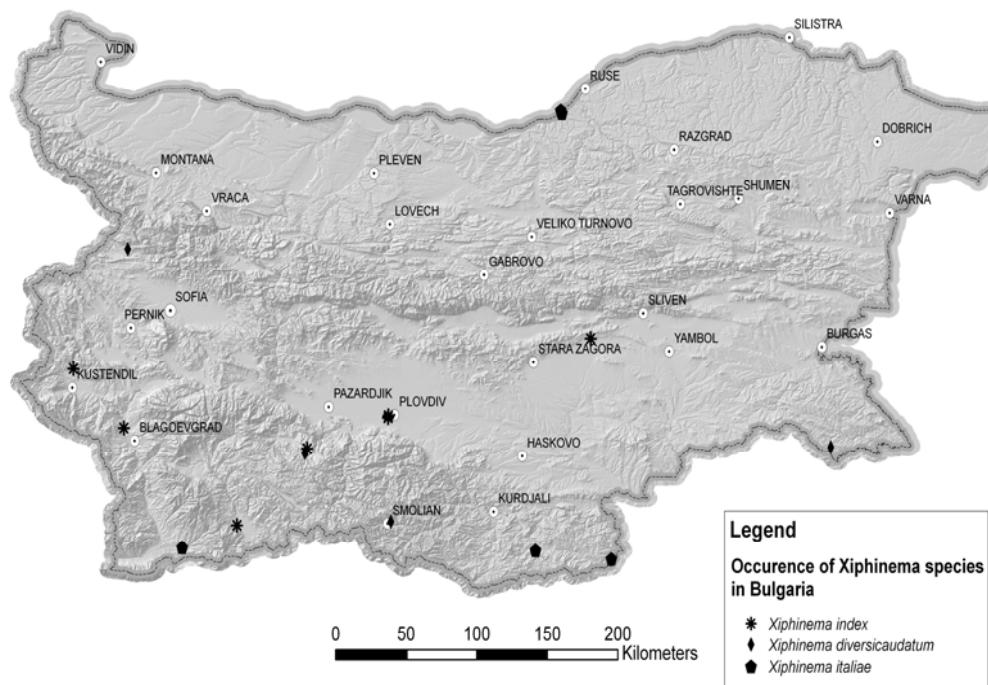


Fig. 3. Distribution of virus vector nematode species of the genus *Xiphinema* in Bulgaria

some of these records require confirmation e.g. *L. caespiticolae*, *X. ingens* and *X. turcicum* for Bulgaria and *X. basilgoodeyi*, *X. elongatum* and *X. rotundatum* for Slovenia. Among the virus vector species, *X. index*, *X. diversicaudatum* and *L. elongatus* occurred in both countries, *X. rivesi* was found only in Slovenia and *X. italiae*, *L. attenuatus*, *L. macrosoma* and *P. maximus* were reported only from Bulgaria. Here we present the current situation in

Bulgaria and Slovenia concerning the occurrence and distribution of longidorid nematode vectors (Figs 1 – 4) and their associated nepoviruses.

Longidorus attenuatus has been reported from vineyards and black currant in several localities only in Bulgaria (Choleva-Abadjieva, 1975; Katalan-Gateva *et al.*, 1982; Choleva & Budurova, 1983).

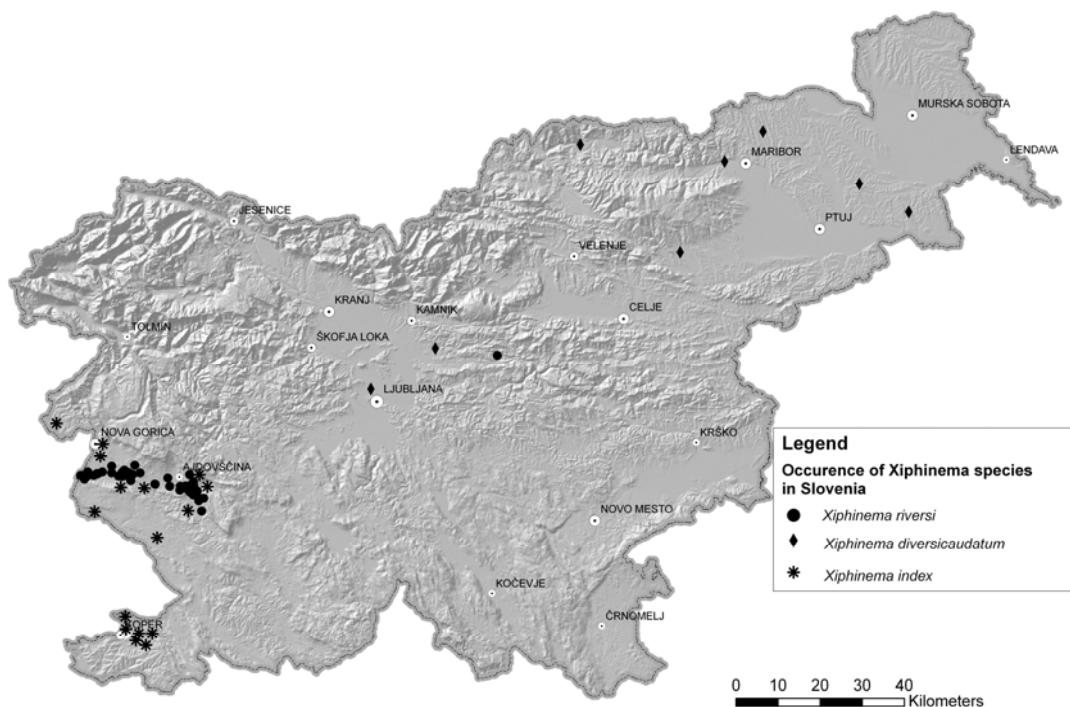


Fig. 4. Distribution of virus vector nematode species of the genus *Xiphinema* in Slovenia

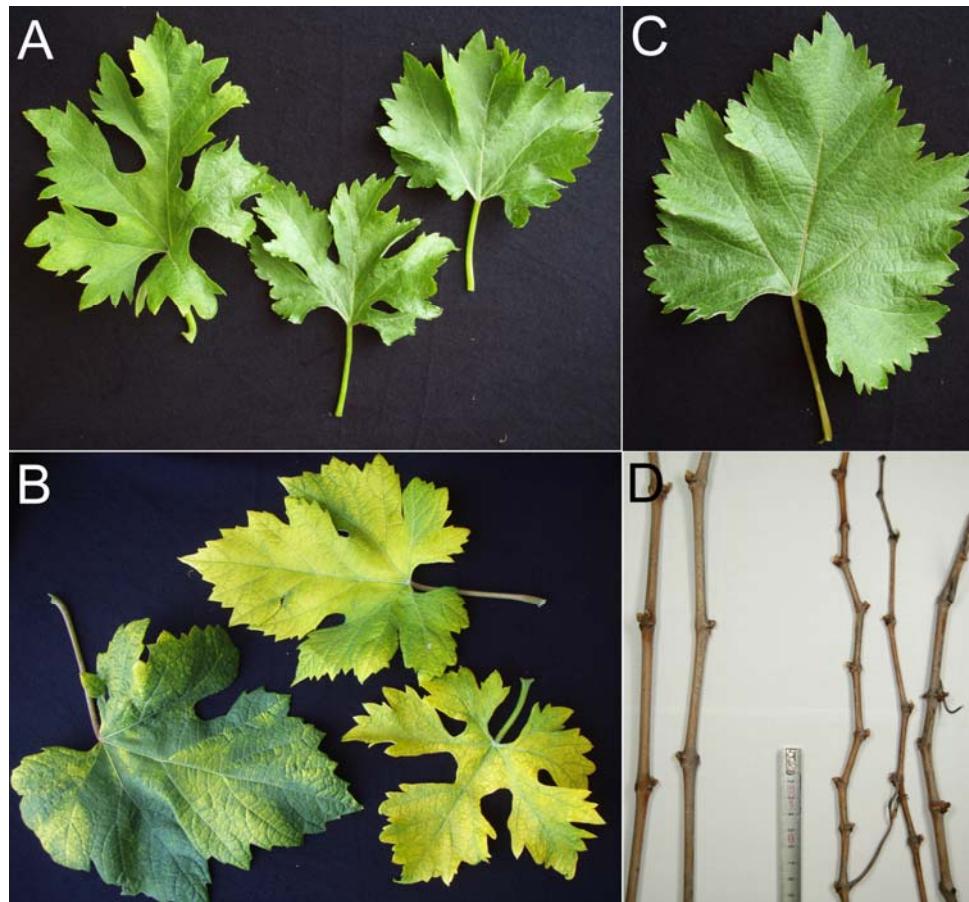


Fig. 5. GFLV infected grapevine cv. “Refošk”: A – fanleaf symptoms on infected leaves, B – different patterns of discolorations on GFLV infected vine, C – healthy vine, D – modification of grapevine “internodium” length, left: healthy vine, right: GFLV infected vine

Longidorus macrosoma - was associated with raspberry in one locality in Bulgaria (Choleva *et al.*, 1992).

Paralongidorus maximus - was found in only one vineyard in Galata (Lamberti *et al.*, 1983).

Xiphinema rivesi - was recovered from several locations in the western part of Slovenia, close to the Italian-Slovenian border (Urek *et al.*, 2003b; Urek *et al.*, 2005) and subsequently was proven as a vector of tobacco ring-spot virus (TRSV) and tomato ring-spot virus (ToRSV) (Širca *et al.*, 2007b).

Longidorus elongatus - is comparatively rare in Bulgaria, reported from vineyards and strawberry fields (Choleva-Abadjieva, 1975; Choleva *et al.*, 1992). In Slovenia it has been reported from vineyards and maize fields, but only in the western part of the country (Urek *et al.*, 2003a).

Xiphinema diversicaudatum - is widespread in both countries in association with various cultivated plants (grapevine, forest trees seedlings, roses, raspberries) and wild vegetation (Choleva & Budurova, 1983; Choleva *et al.*, 1992; Lamberti *et al.*, 1983; Peneva & Choleva 1992; Urek *et al.*, 2003a) and riparian vegetation in Bulgaria (Lazarova *et al.*, 2010).

Xiphinema index - is common in vineyards and orchards in Bulgaria (Stoyanov & Kostadinov, 1975; Choleva-Abadjieva, 1975; Lamberti *et al.*, 1983) and has frequently been found in association with *Salix* spp along rivers (Laz-

arova *et al.*, 2010). This species is reported from vineyards and fig only in the western part of Slovenia (Urek *et al.*, 2003a; Urek & Širca, 2005).

Xiphinema italiae - occurs frequently in Bulgaria associated with different crops, especially grapevine and fruit trees (Stoyanov & Kostadinov, 1975; Choleva-Abadjieva, 1975; Lamberti *et al.*, 1983; Peneva & Choleva, 1992b), also with river bank vegetation (Lazarova *et al.*, 2010).

Longidorus juvenilis - was recorded from two sites in the north-eastern part of Slovenia in association with grapevine (Širca *et al.*, 2007). Recently, Raspberry Bushy Dwarf Virus (RBDV) was detected from different grapevine varieties in Slovenia and also was recovered from *L. juvenilis* specimens soon after they were collected in the field as well as after 4 and 8 months of storage of infested soil in a refrigerator (Mavrič Pleško *et al.*, 2009). The role of *L. juvenilis* in virus transmission, however, is not clear, yet and is still under investigation.

Several nepoviruses (Table 1) have been reported from Bulgaria (Martelli, 1993.; Kamenova *et al.*, 2007; Yankulova *et al.*, 2007; EPPO data). Currently, the only report of a relationship between *X. index* and Grapevine fanleaf virus in Bulgaria refers to transgenic grapes tolerance to the same virus (Tsvetkov *et al.*, 2003). Recently, GFLV was detected in *X. italiae* specimens originating from a

grapevine in Bulgaria, (Širca *et al.*, 2010).

Several nepoviruses have been reported from Slovenia (Table 1, Pompe *et al.*, 2007; EPPO data), but despite an evident relationship between GFLV and *X. index* in several vineyards, only the transmission of tobacco and tomato ring-spot nepovirus (TRSV) and (ToRSV) to bait plants by a Slovenian population of *X. rivesi* has been verified in the laboratory (Širca *et al.*, 2007b). However, these two viruses do not occur in Slovenia (Geric Stare *et al.*, 2009). Nepoviruses ArMV and GFLV, and the Idaeovirus RBDV are frequently found in grapevine production areas throughout Slovenia (Geric Stare *et al.*, 2009) and GFLV infection can be recognized by distinct symptoms (Fig 5) in grapevine varieties.

A wide range of horticultural and agricultural crops are affected by longidorid nematodes and their associated viruses but these virus and crop interactions have not yet been sufficiently studied in Slovenia and Bulgaria. Consequently, more extended research on longidorid nematodes and their associated nepoviruses is now being conducted in these two countries in order to develop and establish control strategies for reducing the detrimental impact of these pathogens on agriculture and horticulture.

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References

- ABRANTES, I. M. DE O., SANTOS, M. S. N. DE A. (1997): General principles of nematode identification. In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS, R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 113 – 127
- ALPHEY, T. J. V., TAYLOR, C. E. (1986): European atlas of the Longidoridae and Trichodoridae. Scottish Crop Research Institute, Dundee, 123 pp.
- ARIAS, M., BRAVO, M. A. (1997): Identification of genera and species in subfamily Longidorinae. In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 128 – 176
- BROWN, D. J. F., BOAG, B. (1997): Sampling for virus vector nematodes. In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 69 – 96
- BROWN, D. J. F., HALBRENDT, J. M. (1997): Identification of *Xiphinema* species (Nematoda: Dorylaimoidea). In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS, R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 177 – 223
- BROWN, D. J. F. WEISCHER, B. (1998): Specificity, exclusivity and complementarity in the transmission of plant viruses by plant parasitic nematodes: an annotated terminology. *Fundam. Appl. Nematol.*, 21: 1 – 11
- CADMAN, C. H. (1963): Biology of soil-borne viruses. *Annu. Rev. Phytopathol.*, 1: 143 – 142
- CATALANO, L., SAVINO, V., LAMBERTI, F. (1992): Presence of grapevine fanleaf nepovirus in populations of longidorid nematodes and their vectoring capacity. *Nematol. Mediterr.*, 20: 67 – 70
- CHOLEVA-ABADJIEVA, B. (1975): [Study on the species composition and distribution of nematodes of the family Longidoridae (Nematoda, Dorylaimoidea) on the vine in Bulgaria.] *Acta Zool. Bulg.*, 3: 19 – 30
- CHOLEVA, B., BUDUROVA, L. (1983): [Parasitic nematode species on black current (*Ribes nigrum* var *europaeum* L.).] *Acta Zool. Bulg.*, 21: 67 – 77
- CHOLEVA, B., PEYKOVA, Y., NEDELCHEV, S. (1992): Study of distribution of nematode virus vectors (Fam. Longidoridae) of raspberry crops in Bulgaria. *Khelminthologiya*, 32: 5 – 10
- COHN, E., TANNE, E., NITZANY, F. E. (1970): *Xiphinema italiae*, a new vector of grapevine fanleaf virus. *Phytopathology*, 60: 181 – 182
- COOMANS, A., HUYS, R., HEYNS, J., LUC, M. (2001): Character analysis, phylogeny and biogeography of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae). *Annales Sciences Zoologiques, Muse'e Royal de l'Afrique Centrale, Tervuren*, 287: 1–239
- DE LUCA, F., REYES, A., GRUNDER, J., KUNZ, P., AGOSTINELLI, A., DE GIORGI, C., LAMBERTI, F. (2004): Characterization and sequence variation in the rDNA region of six nematode species of the genus *Longidorus* (Nematoda). *J. Nematol.*, 36: 147 – 152
- DIGIARO, M., ELBEAINO, T., MARTELLI, G. P. (2007): Development of degenerate and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A, B and C. *J. Virol. Methods*, 141 (1): 34 – 40
- DUARTE, I. M. N., BROWN, D. J. F. (1997): Sampling, detection and identification of nematode transmitted viruses. In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS, R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 313 – 355
- GERIC STARE, B., KNAPIČ, M., MAVRIČ, I., MODIĆ, Š., MUNDA, A., ŠIRCA, S., ŠKERLAVAJ, V., UREK, G., URBANIČIĆ, Z. M., VIRŠČEK MARN, M., ZIDARIČ, I., ŽERJAV, M. (2009): [Technical report of expert work on plant protection for 2009]. Ljubljana, Kmetijski inštitut Slovenije, 2009, pp. 122
- HARRISON, B. D. (1961): Soil borne viruses - tobacco rattle virus. Annual report of Rothamsted Experimental Station for 1961, England, 118 pp.
- HARRISON, B. D. (1964): Specific nematode vectors for serologically distinctive forms of raspberry ringspot and tomato black ring viruses. *Virology*, 22: 544 – 550
- HE, Y., SUBBOTIN, S. A., RUBTSOVA, T. V., LAMBERTI, F.,

- BROWN, D. J. F., MOENS, M. (2005): A molecular phylogenetic approach to Longidoridae. *Nematology*, 7: 111 – 124
- HEWITT, W. B., RASKI, D. J., GOHEEN, A. C. (1958): Nematode vector of soil borne virus of grapevines. *Phytopathology*, 48: 586 – 595
- HOOPER, D. J. (1986): Extraction of free-living stages from soil. In: *Laboratory methods for work with plant and soil nematodes* (Ed. by Southey, J.F.), pp. 5-30. Reference Book, Ministry of Agriculture, Fisheries and Food No. 402. Her Majesty's Stationery Office, London, UK.
- HUBSCHEN, J., KLING, L., IPACH, U., ZINKERNAGEL, V., BROWN, D. J. F., NEILSON, R. (2004): Development and validation of species-specific primers that provide a molecular diagnostic for virus-vector longidorid nematodes and related species in German viticulture. *Eur. J. Plant Pathol.*, 110: 883 – 891
- HUNT, D. (1993): Aphelenchida, Longidoridae and Trichodoridae: their systematics and bionomics. CAB International, Wallingford, 352 pp.
- KAMENOVA, I., TSVETKOV, I., ATANASOV, A. (2007): Virus testing of certified grapevine planting material in Bulgaria. *Biotechnology and Biotechnological Equipment*, 21: 66 – 68
- KATALAN-GATEVA, S., BUDUROVA, L., CHOLEVA, B. (1982): [Phytonematode fauna of the grapevine (*Vitis vinifera* L) from district of Blagoevgrad.] *Annuaire de L'Universite de Sofia "Kliment Ohridski", Livre 1- Zoologie*, 70: 55 – 61
- LAMBERTI, F., CHOLEVA, B., AGOSTINELLI, A. (1983): Longidoridae from Bulgaria (Nematoda: Dorylaimida) with descriptions of three new species of *Longidorus* and two new species of *Xiphinema*. *Nemat Mediterr.*, 11: 49 – 72
- LAMBERTI, F., MOLINARI, S., MOENS, M., BROWN, D. J. F. (2000): The *Xiphinema americanum* group. I. Putative species, their geographical occurrence and distribution. *Russian Journal of Nematology*, 8: 65 – 84
- LAZAROVA S., DE LUCA F., PENEVA, V. 2008. On two closely related species of the *Xiphinema americanum*-group: *X. simile* Lamberti, Choleva et Agostinelli, 1983 and *X. parasimile* Barsi et Lamberti, 2004 (Longidoridae), with a description of the male of *X. parasimile*. *ZooKeys* 3, 29 – 50.
- LAZAROVA, S., ELSHISHKA, M., GROZA, M., PENEVA, V. (2010): Nematodes of Longidoridae and Trichodoridae from riparian habitats in Bulgaria. *Proceedings of the 30th ESN Symposium*, Vienna, 19 – 23 September 2010, p. 127
- LOOF, P. A. A., CHEN, Q. (1999): A revised polytomous key for the identification of species of the genus *Longidorus* Micoletzky, 1922. Supplement 1. (Nematoda: Dorylaimida). *Nematology*, 1: 55 – 59
- MACFARLANE, S. A., NEILSON, R., BROWN, D. J. F. (2002): Nematodes. *Advances in Botanical Research*, 36: 169 – 198
- MARTELLI, G. P. (1993): Graft-transmissible diseases of grapevines. Handbook for detection and diagnosis. FAO, Rome, Italy, 263 pp.
- MINCHEVA, Y., LAZAROVA, S., PENEVA, V. (2008): *Xiphinema pirinense* n. sp. (Dorylaimida: Longidoridae), a new species with a digitate tail from Bulgaria. *Syst. Parasitol.*, 70: 215 – 222
- PENEVA, V., CHOLEVA, B. (1992): Nematodes of the family Longidoridae from forest nurseries in Bulgaria. II. Genus *Xiphinema* Cobb, 1913. *Khelminthologyia*, 32: 47 – 66
- PENEVA, V., LAZAROVA, S., GROZA, M., BROWN, D. J. F. (2005): Diversity of fam. Longidoridae (Nematoda) in Bulgaria. *Abstracts of the Seventh National Conference of Prasitology*, 22 – 25 September 2005, p. 99
- MAVRIC PLEŠKO, I., MARN, M. V., ŠIRCA, S., UREK, G. (2009): Biological, serological and molecular characterisation of *Raspberry bushy dwarf virus* from grapevine and its detection in the nematode *Longidorus juvenilis*. *Eur. J. Plant Pathol.*, 123 (3): 261 – 26
- PLOEG, A. T., BROWN, D. J. F. (1997): Extraction of virus vector nematodes. In: SANTOS, M. S. N. DE A., ABRANTES, I. M. DE O., BROWN, D. J. F., LEMOS R. M. (Eds) *An introduction to virus vector nematodes and their associated viruses*. Instituto do Ambiente e Vida, Coimbra, Portugal, pp. 97 – 112
- POMPE NOVAK, M., GUTIERREZ-AGUIRRE, I., VOJVODA, J., BLAS, M., TOMAŽIČ, I., VIGNE, E., FUCHS, M., RAVNIKAR, M., PETROVIČ, N. (2007): Genetic variability within RNA2 of grapevine fanleaf virus. *Eur. J. Plant Pathol.*, 117: 307 – 312
- SEINHORST, J. M. (1959): A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4: 67–69
- ŠIRCA, S., UREK, G. (2004): The occurrence of *Xiphinema* species in Slovenia. In: European Society of Nematologists XXVII International Symposium: Rome, 14 – 18 June 2004: *Programme and Abstracts*, p 62
- ŠIRCA, S., GERIC STARE, B. MAVRIČ PLEŠKO, I., MARN, M. V., UREK, G. (2007a): First record of *Longidorus juvenilis* and *L. leptocephalus* (Nematoda: Dorylaimida) in Slovenia and their morphometrical and ribosomal DNA sequence analysis. *Russ. J. Nematol.*, 15 (1): 1 – 8
- ŠIRCA, S., STARE, B. G., MAVRIČ PLEŠKO, I., MARN, M. V., UREK, G., JAVORNIK, B. (2007b): *Xiphinema rivesi* from Slovenia transmit *Tobacco ringspot virus* and *Tomato ringspot virus* to Cucumber Bait Plants. *Plant Dis.*, 91 (6): 770
- ŠIRCA, S., UREK, G. (2009): Morphological and molecular characterization of six *Longidorus* species (Nematoda: Longidoridae) from Slovenia. *Russ. J. Nematol.*, 17 (2): 95 – 105
- ŠIRCA, S., ELSHISHKA, M., UREK, G., LAZAROVA, S., MAVRIČ PLEŠKO, I., MARN, M. V., PENEVA, V. (2010): Detection of Grapevine fanleaf virus (GFLV) in *Xiphinema italiae* Meyl, 1953 (Longidoridae) from Bulgaria. *Proceedings of the 30th ESN Symposium*, Vienna, 19 – 23 September 2010, 113
- STOYANOV, D., KOSTADINOV, A. (1975): [Distribution of some parasitic nematodes of family Longidoridae in vineyards in Bulgaria.] *Lozarstvo i Vinarstvo*, 24: 16 – 20
- TAYLOR, C. E., BROWN, D. J. F. (1997): Nematode vectors of plant viruses. CAB International, Wallingford, UK, 286 pp.

- TRUDGILL, D. L., BROWN, D. J. F., McNAMARA, D. G. (1983): Methods and criteria for assessing the transmission of plant viruses by longidorid nematodes. *Rev. Nematol.*, 6: 133 – 141
- TSVETKOV, I., CHOLEVA, B., YANKULOVA, M., COLOVA, V., ATANASOV, A. (2003): Evaluation of transgenic grapes tolerance toward grapevine fanleaf virus. *Proceedings of the 14th ICVG Conference*, 231 – 232
- UREK, G., ŠIRCA, S., KARSSSEN, G. (2003a): A review of plant-parasitic nematodes in Slovenia. *Nematology*, 5: 391 – 403
- UREK, G., ŠIRCA, S., KOX, L., KARSSSEN, G. (2003b): First report of the dagger nematode *Xiphinema rivesi*, a member of the *X. americanum* group, from Slovenia. *Plant Dis.*, 87(1): 100
- UREK, G., ŠIRCA, S. (2005): Longidoridae species from Slovenian vineyard soils. *Lectures and papers presented at the 7th Slovenian Conference on Plant Protection*, Zreče, Slovenia, 8 – 10 March 2005, DRSV 2005, 356 – 359
- UREK, G., ŠIRCA, S., KARSSSEN, G. (2005): Morphometrics of *Xiphinema rivesi* Dalmasso, 1969 (Nematoda: Dorylaimida) from Slovenia. *Russ. J. Nematol.*, 13: 13 – 17
- WANG, S., GERDERICH, R. C. (1998): Immunofluorescent localization of tobacco ringspot nepovirus in the vector nematode *Xiphinema americanum*. *Phytopathology*, 88: 885 – 889
- YANKULOVA, M., TSVETKOV, I., KAMENOV, I., KONDAKOVA, V., VULCHEV, V. (2007): Grapevine virus disease in Bulgaria. *Lozarstvo i Vinarstvo*, 56(1): 19 – 24
- YE, W. M., ROBBINS, R. T. (2004): Cluster analysis of *Longidorus* species (Nematoda: Longidoridae), a new approach in species identification. *J. Nematol.*, 36: 207 – 218
- YE, W. M., SZALANSKI, A. L., ROBBINS, R. T. (2004): Phylogenetic relationships and genetic variation in *Longidorus* and *Xiphinema* species using ITS1 sequence of ribosomal DNA. *J. Nematol.*, 36: 14 – 19

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