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Report on the survey for *Bursaphelenchus xylophilus* and the occurrence of other *Bursaphelenchus* species in Hungarian coniferous forests

Á. Tóth¹ and M. Elekes²

¹Research Centre for Natural Sciences, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025, Budapest, (Hungary); e-mail: toth.agnes@ttk.mta.hu

²Central Agricultural Office, Directorate of Plant Protection and Soil Conservation, Central Laboratory for Pest Diagnosis, Budaörsi út 141-145, H-1118, Budapest, (Hungary)

An ongoing official survey to detect the pine wood nematode *Bursaphelenchus xylophilus*, a quarantine pest, started in 2003 in coniferous forests in Hungary. Based on the results of the study from 2003–11, *B. xylophilus* has not yet been detected in Hungary. Two other *Bursaphelenchus* species (*B. mucronatus* and *B. vallesianus*) were identified in samples in 2009. Details of the survey and the measurements of *B. mucronatus* and *B. vallesianus* are provided.

Introduction

The pine wood nematode, *Bursaphelenchus xylophilus* Nickle, 1970, is a quarantine pest for the European Union (Directive 2000/29/EC) and an EPPO A2 pest recommended for regulation as a quarantine pest. This nematode has been identified as the main agent of pine wilt disease (Kiyohara & Tokushige, 1971). Seasonal drought and high temperatures cause water stress in host trees and play an important role in the development of the disease (Evans *et al.*, 2008). Other species of the genus *Bursaphelenchus* (mycophagous, phytophagous or those with combined feeding habits) have also been considered potential risks to cultivated and native conifers. *Bursaphelenchus xylophilus*, now introduced to Portugal (Roques *et al.*, 2009), is one of the 1296 non-endemic terrestrial invertebrate species in Europe. The most important entry points from which the nematode is thought to have spread were located primarily in Eastern and Northern Europe. Further introduction of potentially harmful nematode species to Hungary, Slovakia, Russia, Poland, Croatia, Sweden or Germany may result in the nematode spreading over more than 50% of the area of these countries by 2030 (Robinet *et al.*, 2011). Since 2003, surveys for *B. xylophilus* have been conducted in Hungary in coniferous forests (Scotch fir, Austrian pine, spruce and other pine) and at potential sources of entry (wood depots, airports, border posts, international transport lines) within a 25 km radius around pine forests.

Description of survey methods and results

The total area of forest in Hungary is 2 million ha, of which 220 000 ha are the host species of *B. xylophilus*. The Nematological Laboratory of the Central Laboratory for Pest Diagnosis (CLPD), Central Agriculture Office, Directorate of

Plant Protection and Soil Conservation has been conducting an ongoing survey since 2003 in coniferous forests and at potential sources of entry for *B. xylophilus* within a 25 km radius around pine forests. The survey, focusing primarily on *Pinus sylvestris* and *P. nigra* forests where trees displayed symptoms of damage by Cerambycid beetles or by other pests or pathogens (Tóth *et al.*, 2012), is carried out in all Hungarian counties in cooperation with the official forestry and plant health services, and follows the methods suggested by CLPD. Morphological and molecular diagnostic tests used follow international protocols (EPPO, 2009, 2011). *Bursaphelenchus xylophilus* and other European *Bursaphelenchus* species were not detected in plant samples between 2003 and 2008. Specimens of *Aphelenchoides* spp., *Laimaphelenchus* sp. and *Tylenchina* suborder were identified in addition to saprophytic nematodes in wood samples. The number of samples per year varied between 120 and 260 (Table 1). In 2009, for example, 305 wood samples from the whole territory of the country were analysed by the Nematological Laboratory. According to the national survey, the conifer samples comprised 78% *P. sylvestris*, 12.5% *P. nigra*, 6.2% *Picea abies* and 3.3% other species (*Abies alba*, *Larix decidua*, *Pinus strobus*).

As described previously (Tóth & Elekes, 2011), nematodes collected were identified to species level by comparing their microscopic images with the morphological and morphometric characteristics of *Bursaphelenchus* males and females (Table 1). *Bursaphelenchus mucronatus* (Mamiya & Enda, 1979) was first detected in Hungary in conifer samples in 2009. *Bursaphelenchus vallesianus* (Braasch *et al.*, 2004), a species new to the Hungarian national fauna, was identified from wooden chip samples from Veszprém county.

As well as signs of insect damage (Scolitydae, Cerambycidae), symptoms of the blue stain fungus (*Ceratosomella*) were observed on a windbreak *P. sylvestris* stand in

Table 1 Results of the *Bursaphelenchus xylophilus* survey in Hungary between 2003 and 2011

Year	Number of locations	Number of samples	<i>Bursaphelenchus</i> species in samples
2003	71	123	–
2004	82	130	–
2005	88	144	–
2006	96	142	–
2007	147	200	–
2008	153	262	–
2009	170	305	<i>B. mucronatus</i> , <i>B. vallesianus</i>
2010	173	252	<i>B. mucronatus</i> , <i>B. vallesianus</i>
2011	143	165	<i>B. mucronatus</i> (source: CLPD report)

North-Western Hungary, near Káptalanfa, while collecting the obligatory amount (100–150 g) of wood shavings in 2009. In these samples, *B. mucronatus* was identified in high numbers (approximately 30 000 individuals) along with nearly 5000 saprophytic Rhabditidae. *Bursaphelenchus mucronatus* was detected in the samples collected from dying trees and also from nearby asymptomatic *P. sylvestris* trees. In North-Western Hungary, near Tótvázsony, the 100–150 g wood shavings were collected from a *P. nigra* forest. The stand showed signs of insect and fungal infection, and 40 individuals of *B. vallesianus* and several hundred pieces of saprophytic Rhabditidae were found in the wood samples. *Bursaphelenchus mucronatus* was detected in the same part of the forest in September, during repetitive sampling. Under the bark of the dead, dry pines, larvae and adults of *Rhagium inquisitor* (Linnaeus, 1758) were found in pupal chambers.

In 2010, 252 samples were collected from the whole territory of the country in the framework of the official survey. There were 221 individual samples from a total of 146 sampling locations, with 31 samples collected from areas defined as endangered forests (27 locations). Of the coniferous wood chip samples, 49% belonged to *P. sylvestris*,

31% to *P. nigra*, 17% to *Picea abies* and 3% to *Larix decidua*. Samples from the endangered forest area displayed the following species composition: *P. sylvestris*, 59%; *P. nigra*, 32%; *Picea abies*, 6%; *Pinus strobus*, 3%. On every *Bursaphelenchus*-infested tree, the blue stain fungus was also present. Two *Bursaphelenchus* species were identified: *B. mucronatus* and *B. vallesianus*.

According to the NEMATOL GIS System, male and female adults of *B. mucronatus* have already been detected by foreign researchers in deciduous forests in Central Hungary, in the vicinity of settlements Tass and Szalkszentmárton. In 2009, CLPD did not find coniferous host plants of *Bursaphelenchus* in this location (based on GPS coordinates). This means that, regardless of the earlier published presence of *B. mucronatus*, the species can no longer be found in this area. Schauer-Blume and Sturhan (1989) detected *B. fraudulentus* in 1989 on *Quercus petraea* near the Austro-Hungarian border in Sopron. The samples they obtained were used for molecular studies.

Conclusions

According to the official survey, *B. xylophilus* was not detected between 2003 and 2011 in Hungary. The results confirm that Hungary is free from *B. xylophilus* (status: absent). The presence of *Monochamus* vector species in Hungary increases the likelihood of *B. xylophilus* establishing and spreading in Hungary.

Rapport sur la prospection pour la détection de *Bursaphelencus xylophilus* et la présence d'autres espèces de *Bursaphelenchus* dans les forêts de conifères hongroises

Une prospection officielle encore en cours a débuté en 2003 dans les forêts de Hongrie pour détecter le nématode du pin, *Bursaphelenchus xylophilus*. Sur la base des

Table 2 Measurements of *Bursaphelenchus mucronatus* and *B. vallesianus* (Tóth & Elekes, 2011)

Parameter	Species			
	<i>B. mucronatus</i> (Hungary, Káptalanfa)	<i>B. mucronatus</i> Brzeski & Baujard, (1997)	<i>B. vallesianus</i> (Hungary, Tótvázsony)	<i>B. vallesianus</i> Braasch et al., (2004)
Tail length (mm) ♂	0.72	0.70 (0.48–1.03)	0.71	0.75 (0.51–1.05)
a	36.60	41.3 (24.5–56.0)	40.88	38 (22–48)
b	6.97	10.0 (6.6–14.7)	11.44	10 (7.8–14.1)
c	29.29	24.7 (14.6–35.6)	35.77	29 (20–37)
Stylet (µm)	12.5–15.0	13.4 (11.5–17.5)	12.5	13 (12–15)
Spicules (µm)	25.70	25 (16–32)	17.5	16 (14–19)
Tail length (mm) ♀	0.71	0.78 (0.56–1.05)	0.71	0.83 (0.57–1.08)
a	36.20	39.5 (25–51)	36.50	39 (30–47)
b	7.24	10.6 (6.1–15.9)	9.23	11 (9.1–12.99)
c	31.50	25.1 (16.9–35)	35.0	34 (26–43)
Stylet (µm)	13.75–15.0	13.8 (12–16)	11.25–13.75	13 (11–15)
Vulva (%)	76.00	73.2 (66–78)	74.00	73 (71–76)

résultats de l'étude de 2003–2011, *B. xylophilus* n'a pas encore été détecté en Hongrie. Deux autres espèces de *Bursaphelenchus* (*B. mucronatus* et *B. vallesianus*) ont été identifiées dans des échantillons en 2009. Les détails de la prospection, ainsi que les mesures réalisées sur *B. mucronatus* et *B. vallesianus* sont fournis.

Отчет об обследовании на выявление *Bursaphelenchus xylophilus* и присутствие других видов *Bursaphelenchus* в хвойных лесах Венгрии

В хвойных лесах Венгрии начиная с 2003 года ведется официальное обследование, направленное на выявление сосновой стволовой нематоды *Bursaphelenchus xylophilus*, карантинного вредного организма. На основе результатов исследования, проводившегося в Венгрии в период с 2003 по 2011 гг., *B. xylophilus* еще не была выявлена. Однако, в пробах древесины в 2009 г. были обнаружены два других вида *Bursaphelenchus* (*B. mucronatus* и *B. vallesianus*). В статье приводятся детальные данные обследований и замеры *B. mucronatus* и *B. vallesianus*.

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