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Hunting Nematodes in the Pine Forests of Northern Greece: A Preliminary Overview after One Year of Surveys †

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Abstract: Pine wood nematode (PWN) *Bursaphelenchus xylophilus* is one of the most important forest tree pathogens worldwide, causing Pine Wilt Disease. Facilitated by international trade, PWN has expanded beyond its natural range becoming one of the most notorious quarantine pests. To prevent its further expansion, national survey programs are being widely established. Accordingly, such a network has also been established in Greece collecting and examining wood samples nationwide. So far, *B. xylophilus* was absent from all samples examined, although four other *Bursaphelenchus* species were identified, along with other nematode taxa. Evidently, besides preventing the introduction of *B. xylophilus*, national survey programs can further increase our knowledge regarding tree-inhabiting nematodes.

Keywords: *Bursaphelenchus* spp.; national survey program; nematodes; conifers; Greece

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1. Introduction

The Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970 is one of the most important pathogens of high economic importance worldwide, causing the Pine Wilt Disease. The PWN has managed to expand its range following the routes and pathways employed by the international trade. Coupled with the detrimental impact it has on pine forests [1,2], it has been listed as a quarantine pest in more than 40 countries in the EU [3] and considered one of the most important pests and pathogens in the world [2].

PWN can be easily transferred via wood trade either as a commodity (e.g., live plants, wood logs, sawn wood), or as Wood Packaging Material (WPM) [4]. It was introduced to Eastern Asia and Western Europe by human activities and international trade [5]. It was first recorded in Japan at the beginning of the twentieth century [6,7] where it was probably transferred with timber exported from North America and has caused significant damages in pine forests since then. *Bursaphelenchus xylophilus* was also introduced to China, Taiwan, and Korea where it was detected in mid to late 1980s [8]. In Europe, it was reported for the first time in Portugal in 1999 [9] and in 2008 the first report of *B. xylophilus* was confirmed in Spain [10].

Since its introduction into new regions, it has become evident that the impact of *B. xylophilus* is more pronounced in the areas where it has been introduced compared to its natural range such as North America, Canada and the United States [11], and Mexico [12]. In fact, it has been noticed that while North American pine species are resistant or at least

tolerant to *B. xylophilus*, exotic species such as *P. nigra*, *P. sylvestris*, and *P. thunbergii*, planted in North America, especially in the warmer southern areas of the United States, are most susceptible [8,12,13].

Since *B. xylophilus* expansion and its establishment outside its natural range there have been reports of many damaging effects on forest ecosystems and simultaneous major economic impacts [14,15] while further future and even higher economic impacts are assumed to be expected [16]. After its first detection in Portugal and Spain, it has become evident that PWN poses a major threat for the pine forests along the Mediterranean basin. This becomes apparent considering the large extent of pine forests [17], the favorable climatic conditions [18] in combination to climate change scenarios [19] and the occurrence of its vector *Monochamus galloprovincialis* (Olivier 1795) in many countries [20,21]. To avoid its further expansion, national survey programs screen several samples from trees showing symptoms similar to the ones caused by PWN all over Europe. In this direction, some results from Greece are presented here, emphasizing on the fact that beside preventing the introduction of *B. xylophilus*, surveys can substantially contribute to our knowledge on the nematode species inhabiting pine trees in Greece.

2. Materials and Methods

In the framework of the National Survey Program regarding the PWN, wood samples were collected from northern and central Greece and the northern Aegean islands, and then examined at the Forest Research Institute in Thessaloniki. Forty-five (45) wood samples in total were collected from declining or symptomatic conifer trees. Samples were processed immediately after arriving at the laboratory. Nematodes were extracted using a modified Baermann funnel technique. For each sample, approximately 10 gr of wood chips were soaked in water for at least 24 hours at room temperature. A binocular stereoscope was then used for the detection of nematode presence while individual nematodes were picked with a micropipette and mounted on a glass slide for further identification under a microscope. Nematode identification was made based on their morphological characteristics [11,22].

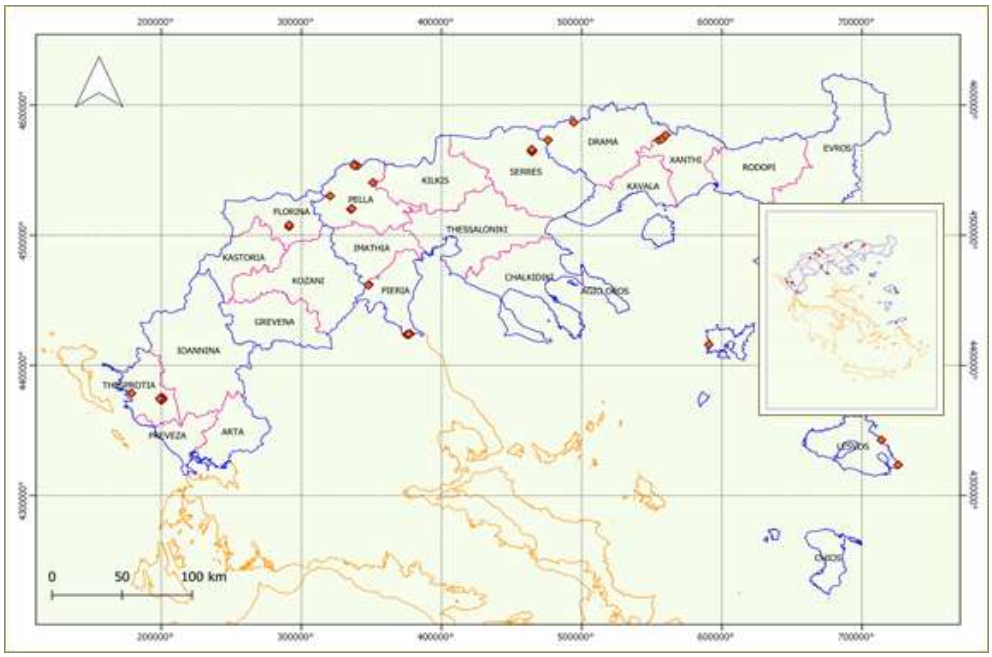

Figure 1. Sampling sites covering north and central Greece and part of the northern Aegean islands.

3. Results

Out of the 45 samples examined (Figure 1), nematodes were detected in 13 samples, i.e., 29% of the samples. Even though *B. xylophilus* was not detected in any of the 13 samples, other *Bursaphelenchus* spp. were detected in 8 of them (62%). From these 8 samples, all samples contained only one *Bursaphelenchus* species (87% of the samples), except for one sample containing two *Bursaphelenchus* species. A large number of samples (69% - 9 samples) also contained other nematode taxa besides *Bursaphelenchus* species. In particular, four *Bursaphelenchus* species were identified: *B. hellenicus* Skarmoutsos, Braasch, Michalopoulou 1998, *B. mucronatus* Mamiya and Enda 1979, *B. leoni* Baujard 1980 and *Bursaphelenchus* sp.1. There were also some *Bursaphelenchus* sp. individuals that could not be assigned to one particular species (Table 1). The fact that, either individuals were at a very immature stage, or in a bad condition after the extraction, made any further identification to species level very difficult. All *Bursaphelenchus* species were encountered with the same frequency.

Regarding the other nematode species that were detected (Table 1), they belong to *Aphelenchoides* sp., *Tylencholemaillus* sp. (fungivores), Anguinidae (fungivores/ plant feeders), *Panagrolaimus* sp. (bacterivores), *Clarkus* sp. (predators) and *Parasitorhabditis* sp. (entomophilic).

Samples with *Bursaphelenchus* species and other nematode taxa are located in three administrative regions: Drama, Pella and Thesprotia, while other nematode taxa were also found in Pieria region in addition to the other three regions (Table 1).

Table 1. Regions, conifer species and detected nematodes.

Regions	Conifer species	*Bursaphelenchus* spp.	Other nematodes
Aridaia	*Abies borisii-regis*	*B. mucronatus*	
	Pinus silvestris	*Bursaphelenchus* sp.1	
Neurokopi	*Pinus silvestris*	*B. hellenicus*	Anguinidae
	Pinus silvestris	*B. leoni*	
	Pinus sp.	*B. mucronatus*	*Parasitorhabditis* sp.
Pella	*Pinus* sp.	*Bursaphelenchus* sp.1	
	Pinus sp.	*Bursaphelenchus* sp.†	*Aphelenchoides* sp.
Pieria	*Pinus nigra*		*Panagrolaimus* sp.
Thesprotia	*Pinus* sp.	*B. hellenicus*	*Tylencholaimellus* sp.
	Pinus sp.	*Bursaphelenchus* sp.†	*Clarkus* sp.
	Pinus sp.		Other nematodes

† *Bursaphelenchus* individuals that could not be identified.

4. Discussion

Bursaphelenchus xylophilus was absent from all samples with nematode presence while other *Bursaphelenchus* spp. were detected in more than half of the samples with nematode detection indicating a strong presence of this genus. The identified *Bursaphelenchus* species were: *B. hellenicus*, *B. mucronatus*, *B. leoni* and *Bursaphelenchus* sp.1. Detection of these species in the current study is validated by previous studies in Greece. In particular, *B. hellenicus*, *B. leoni* and *B. mucronatus*, have already been documented for Greece [23,24]. Regarding the unidentified *Bursaphelenchus* sp.1, it resembles *B. vallesianus*, a member of the "sexdentati" group sensu Braasch [25] and Braasch et al. [22], a species also detected in Greece in specimens isolated by E. Skarmoutsos during 1997 and 1998 and analyzed by Lange et al. [26]. In further support of these findings, most of these species have also been documented in neighboring countries. For example, in Cyprus, apart from *B. leoni* [27,28], *B. borealis*, *B. idius B. sexdentati* and *B. teratospicularis* [28] have also been recovered from trees in a bad condition, wilted or damaged. In Italy besides *B. hellenicus*, *B. mucronatus*, *B. leoni*, *B. sexdentati*, and *B. teratospicularis* [29,30] other members of the *Bursaphelenchus*

genus like *B. abietinus*, *B. andrassyi*, *B. eremus*, *B. fraudulentus*, *B. fungivorous*, *B. minutus* and *B. tusciae* [30–34] have been identified. In Turkey, *B. anamurius*, *B. hellenicus*, *B. mucronatus*, *B. pinophilus*, *B. sexdentati* and *B. vallesianus* have been identified by Akbulut et al. [35–39]: *B. anamurius*, *B. hellenicus*, *B. mucronatus*, *B. pinophilus*, *B. sexdentati* and *B. vallesianus*.

Generally, *B. mucronatus* was long considered the most abundant *Bursaphelenchus* species in Central Europe but relatively rare in the Mediterranean region according to Braasch [25]. Nevertheless, recent studies have shown that *B. mucronatus* is quite frequent around the Mediterranean region and the Balkan Peninsula [40], an issue that was also retrieved in the current study. In contrast, *B. leoni* is referred as a typical Mediterranean species, based on its widespread and frequent occurrence there, although it has also occasionally been found in Central Europe. *Bursaphelenchus hellenicus* besides Greece [41] has also been documented in two other Mediterranean countries such as Italy [30] and Turkey [39]. As for *B. xylophilus* presence in Europe, so far it has been detected only in Portugal [2] and Spain [10,42].

Apart from *Bursaphelenchus* spp., other nematode taxa were also recovered in many samples, something that has also been frequently reported [28,29,37,43,44]. These taxa range from saproxylic and entomophilic, to phytophagous, predatory and fungal-associated nematodes [45]. For example, Caroppo et al. [29] state that Rhabditida and other Aphelenchida occurred in most pine wood samples, while Tylenchida presence was occasional and restricted to a limited number of specimens. Dod et al. [46] found that saprophytic nematodes (Rhabditidae, Diplogasteridae and Cephalobidae) dominated along the entire coastal region of Croatia while *Bursaphelenchus* spp. presence was quite low.

Summarizing, it is easily deduced that in the framework of the national survey programs focusing on *B. xylophilus*, significant knowledge can be gained and accumulated regarding other *Bursaphelenchus* and nematode species as well. It should be made clear that *B. xylophilus* is not the only pathogenic *Bursaphelenchus* species. In Greece for example, among the *Bursaphelenchus* species identified so far, *B. mucronatus*, *B. vallesianus* and *B. sexdentati* have been characterized to be highly pathogenic [43,47,48], although such findings have not been confirmed under natural forest stand conditions [49]. However, such pathogenicity results are indicative of the species pathogenic potential and detection of such *Bursaphelenchus* species could add to the knowledge of the condition pine forests are at present in Greece.

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