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AN OUTLOOK ON THE ASSOCIATION OF *BURSAPHELENCHUS LEONI* WITH WILTING PINES IN CYPRUS

by
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Summary. In Cyprus, *Bursaphelenchus leoni* was found to be associated with pine wilting; 48% of the wood samples examined from seriously wilted pine trees (*Pinus brutia*) were infested with the nematode while the overall mean nematode infestation, including *P. pinea* and *P. nigra*, reached 56 per cent. Very few nematodes were recovered from pine trees showing no clear wilt symptoms. Bark beetle attack was evident in all seriously wilted trees while nematode populations in young trees were higher than in very old trees. *P. nigra* is reported for the first time as a host of *B. leoni* in Cyprus.

In Cyprus, forests occupy 19 per cent of the total area of the island while more than 90 per cent of the area of forest trees consist of the pine species *Pinus brutia* Tenore, *P. nigra* Arnold and *P. pinea* L. Forestation is undertaken in somewhat difficult circumstances because of low precipitation and high summer temperatures. Pathogens and pests are a further stress and therefore any information on the occurrence and pathogenicity of new diseases of forest trees on the island is of paramount importance.

Several species of *Bursaphelenchus* have been found in association with wilted pines in the Mediterranean region (Baujard, 1980; Marinari Palmisano *et al.*, 1991, 1994; Philis and Braasch, 1996) but only *B. xylophilus*, which was not found in Cyprus, has so far been shown to be significant in causing tree mortality. Another closely related species, distributed mainly throughout Europe and Asia, is *B. mucronatus* which is usually not pathogenic, but is suspected to kill pine trees in the Far East of Russia (Kulinich and Kolosova, 1994) or to act somewhat pathogenic under sufficient high temperature as shown in experiments (Schauer-Blume, 1990; Braasch, 1996). Other *Bursaphelenchus* species and their possible vector beetles (Mamiya and Enda, 1972) should also be investigated in relation to the occurrence and spread of pine wilt disease.

The present work describes the findings of a survey of pine trees, undertaken to determine the incidence of *B. leoni* Baujard, in Cyprus forests.

Materials and methods

Tree sampling was carried out from early spring to late autumn, 1995, in forests located in the south east area of the Troodos mountains region. Wood samples were taken from a total of 96 pine trees, mostly *Pinus brutia*, of various ages. Each sample was a core of wood, 8-10 cm long and 0.5 cm diam. and about 6 g weight taken with a pressle borer at a height of 1.5 m above soil level. Samples were placed in airtight plastic bags for transportation to the laboratory where they were cut into pieces 3-4 mm long

and all nematodes present were extracted by the Orbit Environ Shaker method (Philis and Braasch, 1996). Nematode extraction was undertaken for 3 days for each sample and the suspension was then poured into beakers and left to settle for 30 minutes. The excess water was then poured off and the remaining 40-50 ml suspension was placed in 10 ml centrifuge tubes and centrifuged for 8 minutes at 1500 rpm to concentrate the nematodes. Nematodes were counted in 2-3 ml suspension using a stereoscopic microscope at X25 magnification. The sampled trees were classified according to their estimated age and whether they were healthy, "branch wilted" or "overall, seriously wilted", the last category corresponding to the red leaf, completly wilted tree condition (Philis and Braasch, 1996).

Results and discussion

A close correlation was found between seriously wilted pine trees and the presence of *B. leoni*, the overall mean per cent of nematode-infested trees reaching 55.7% (Table I). *Pinus nigra* was also found infested with the nematode, which is the first record of this species as a host of *B. leoni* in Cyprus. Few nematodes were recovered from trees with no clear wilting symptoms, i.e. "branch wilting" (or "flags") (Table I). Although there is still no proof that *B. leoni* causes pine wilting in Cyprus, similar cases were reported by Malek and Appleby (1984) who found that sampling of wood from partially wilted pine trees, caused by *B. xylophilus*, did not reveal any nematodes in the main trunk. During the study it was also observed that resampling, after 6-7 month interval, of some of the most seriously nematode infested trees did not reveal any nematodes from trunk wood. This most probably is because nematode populations, after reaching a maximum level, then decrease gradually as the tree reaches an advanced state of deterioration. Nematode recovery has also shown that young trees tend to harbour more nematodes than old trees (Table II).

Sampled sites included trees that had died one year, or even less, before, showing any evidence of bark beetle activity (Fig. 1). Although *Monochamus* spp., vectors of *B. xylophilus* and *B. mucronatus*, do not exist in Cyprus (Georgiou, 1977), there is a strong possibility-

TABLE I - *Sampled forest areas and presence of* Bursaphelenchus leoni *on pines.*

Area (Fig. 1)	Site No.	Number of trees sampled	Tree appearance and nematode occurrence				Per cent nematode infested trees		Altitude (m)
			Branch wilting	Positive	Overall serious wilting	Positive	Branch wilting	Overall serious wilting	
Limassol	1	3	–	–	3	3*	0.0	100.0	50
Asinou	2	16	15	2	1	1	13.3	100.0	400
Kornos	3	10	2	–	8	5	0.0	62.5	300
Macheras	4	9	6	–	3	1	0.0	33.3	650
Agros	5	9	–	–	9	5	0.0	55.5	1.100
Platres	6	25	7	1	18	6**	14.2	33.3	1.300
Platania	7	5	4	–	1	–	0.0	0.0	1.000
Gefyri-Panayas	8	14	5	–	9	6	0.0	66.6	500
Mandra-Kambion	9	5	1	–	4	2	0.0	50.0	500

* Refers to *Pinus pinea*; ** One of the trees was *P. nigra*.
Sampling of more than fifteen healthy trees (*P. brutia*) did not reveal any nematodes from trunk wood.

TABLE II - *Nematode populations from pine wood of different age.*

Age of trees	Number of trees infested with *B. leoni*		Mean nematode population/g dry wood		Bark beetle attack* (1-3)
	P. pinea	*P. brutia*	*P. pinea*	*P. brutia*	
1-20 yrs	3	1	70	22	2.2
21-40 yrs	–	6	–	25	2.4
41-80 yrs	1	15	20	13	2.3
> 80 yrs	–	3	–	4	2.7

* 1: Trace to light 2: Light to moderate 3: Moderate to serious infestation.

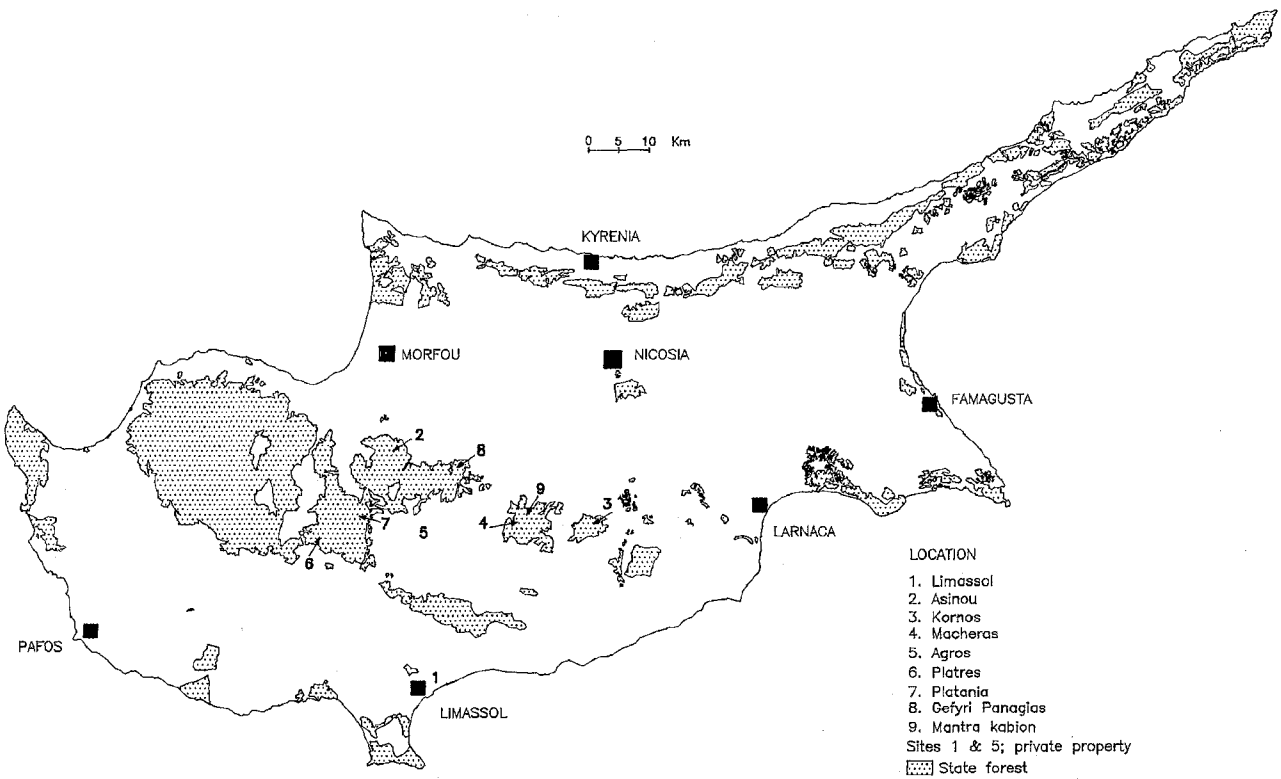

Fig. 1 - Forest map of Cyprus and sampling sites (see Table I).

that bark beetles present on the island may play an important role in transmitting *B. leoni*. Mamiya and Enda (1972) found *Bursaphelenchus xylophilus* also in the body of the long horn beetle *Arhopalus rusticus* Linne and in Cyprus *Arhopalus ferrus* Muls. and *Arhopalus syriacus* Reitter have already been reported (Georgiou, 1977). Bark beetle attack was more pronounced on older than on young trees (Table II) and holes, made by beetles, appeared in different size and shape. On some trees, mostly very old, they were large and oval while on other trees they were round, 3-4 mm diam., with an L-shape tunnel formation (Fig. 2).

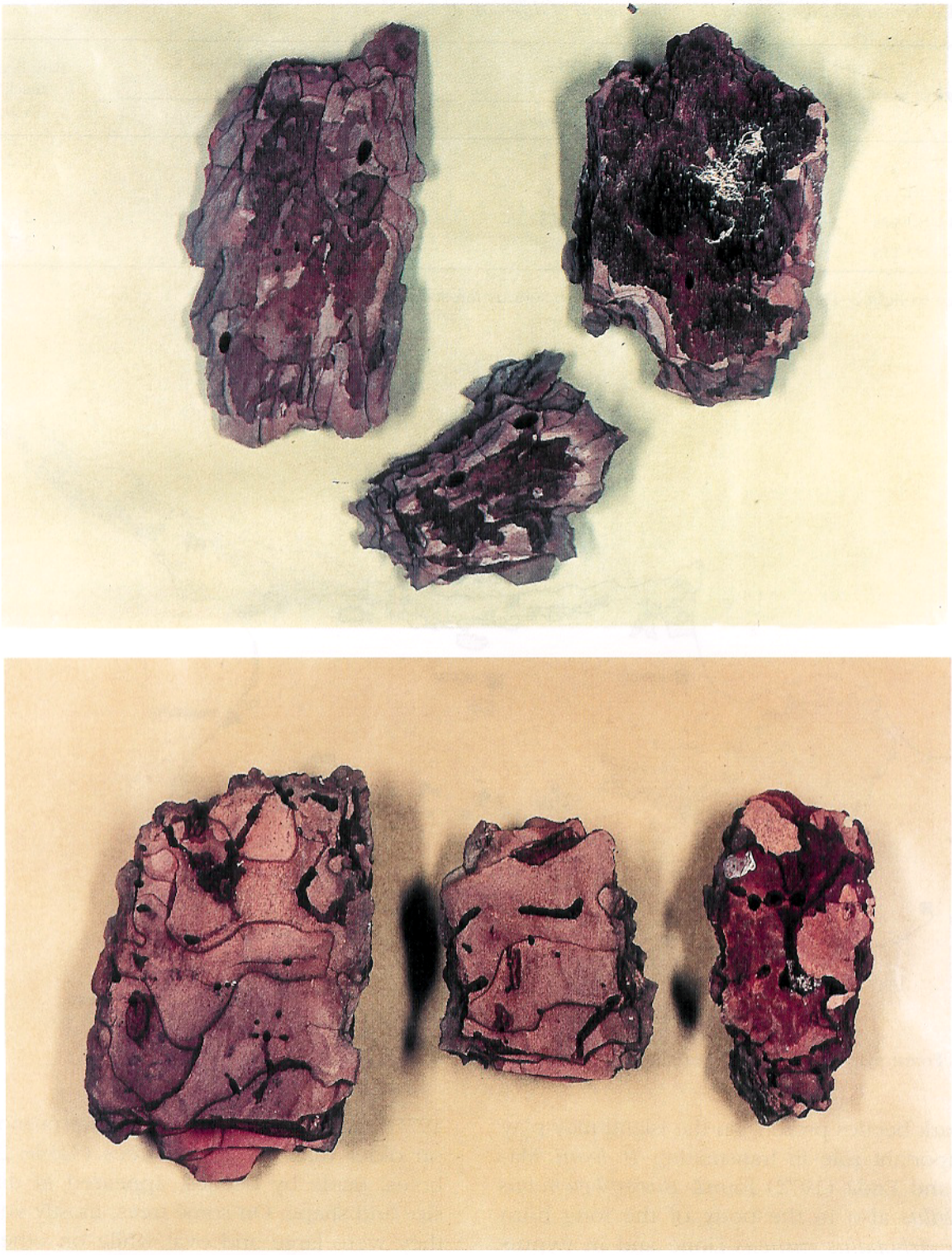

Fig. 2 - Emergence holes of adult beetles infested with *Bursaphelenchus leoni*: oval shape caused by Cerambicidae on very old trees (top) and small and round on younger trees (bottom).

In Japan, greatest forest damage caused by *B. xylophilus* has been correlated with drought-stressed trees and mean air temperatures above 25 °C for 55 days (Takeshita et al. 1975). In Cyprus, the mean August temperatures at Platania and Kornos forest stations ranged between 24 and 27 °C, while similar summer temperatures are expected to occur in the other forest areas of Cyprus. Thus, in the event of *B. xylophilus* and its insect vector being introduced to the island it seems likely that in view of the sustained August temperatures they could become dangerous. At 25 °C the life cycle of *B. xylophilus* is completed in 4 to 5 days (Mamiya, 1984) and it is probable that other *Bursaphelenchus* species would respond to high temperatures. Altitude does not seem to influence the presente of the nematode since infestation occurred at both low (Kornos) and high elevations (Platres).

Research work on the distribution and biology of *Bursaphelenchus* species on forest trees of the Mediterranean region should be intensified in order to clarify their real role in causing pine wilt, as already observed at several places.

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