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# OCCURRENCE OF THE PINE WOOD NEMATODES, *BURSAPHELENCHUS* SPP., AND THEIR POSSIBLE VECTORS IN ITALY[1]

by

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**Summary**. Seven Italian regions were sampled in 1996-97 to investigate on the occurrence of *Bursaphelenchus* species associated with coniferous forests showing decline symptoms of unknown etiology. A total of 68 composite samples were collected in 38 localities from 169 trees of *Pinus pinaster, P. sylvestris, P. halepensis, P. pinea, P. nigra austriaca, P. strobus, Larix decidua* and *Juniperus communis*. *Bursaphelenchus xylophilus* was never detected. Specimens of *B. mucronatus* occurred in eight localities, *B. leoni* in seven, *B. sexdentati* in five and *B. teratospicularis* in three. *Bursaphelenchus* sp., considered to be an undescribed species, was found in the xylem of *P. pinea* in a single locality in Tuscany. Six *B. mucronatus*, two *B. sexdentati* and the *Bursaphelenchus* sp. populations were reared *in vitro* on cultures of *Botrytis cinerea*. *B. teratospicularis* is recorded for the first time in Italy. New host records for Italy are *P. sylvestris, P. nigra austriaca* and *P. strobus* for *B. mucronatus*; *P. sylvestris* for *B. leoni*; *P. pinea* and *P. halepensis* for *B. sexdentati*. No *Bursaphelenchus* specimens were recovered from insects of the genera *Monochamus, Pissodes, Sirex, Rhagium, Acanthocinus, Horthotomicus, Arhopalus* and *Tomicus* captured in the sampled forests.

An investigation was undertaken in 1996-97, as part of a European Union initiative, to establish if the presence of pine wood nematodes, *Bursaphelenchus* spp. are associated with, and the cause of, the decline of coniferous trees observed in Italy.

## Materials and methods

Coniferous forests were selected, with the collaboration of the Regional Forest Service, in the seven regions indicated in Table I. These did not include some previously sampled (Ambrogioni *et al.*, 1994) and where no specimens of *Bursaphelenchus* spp. had been detected.

Declining trees, with symptoms of unknown etiology, or recently dead (within six months) trees with evidence of insect and fungus presence were sampled. Small trees were cut at the base and several pieces of wood were taken along the length of the trunk; samples of large trees were taken from the main branches previously sawn from the trunk. Each sample comprised wood pieces from 1 to 9 different trees and kept in plastic bags in a refrigerator until required for processing.

A total of 68 composite samples were collected from 38 localities (Table I) from 169 trees.

Nematodes were extracted from 30 g samples per tree in a Baermann funnel operated for

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TABLE I - *Localities sampled and insect species detected.*

| Sample No* | Region | Locality (Province) | Tree species | Condition of trees** | Insect captured | |
|---|---|---|---|---|---|---|
| | | | | | Species | No |
| 1/4 | Val d'Aosta | La Côte (AO) | *P. sylvestris* | Insect attack, reddish needles, dying shoot | *Monochamus galloprovincialis galloprovincialis* | 1 |
| 2/1 | Val d'Aosta | La Côte (AO) | *Larix decidua* | Dying shoot | | |
| 3/1 | Val d'Aosta | Brusoncle (AO) | *P. sylvestris* | No damages | | |
| 4/3 | Val d'Aosta | St. Denis (AO) | *P. sylvestris* | Dying trees | | |
| 5/1 | Val d'Aosta | St. Denis (AO) | *Larix decidua* | No damages | | |
| 6/3 | Val d'Aosta | Dajey (AO) | *P. sylvestris* | Dying trees, blue stain | | |
| 11/4 | Liguria | Orco Fellino (SV) | *P. pinaster* | Dead tree, insect attack | *M. g. galloprovincialis*<br>*Pissodes castaneus*<br>*Sirex juvencus* | 2<br>2<br>1 |
| 12/6 | Liguria | Monte Bignone (IM) | *P. pinaster* | Partially damaged | | |
| 13/1 | Liguria | Perinaldo (IM) | *P. pinaster* | Partially damaged | | |
| 14/1 | Liguria | Perinaldo (IM) | *P. pinaster* | Partially damaged | | |
| 15/9 | Liguria | Arenzano (GE) | *P. pinaster* | Dead and dying trees | | |
| 16/7 | Liguria | Prati di Prà (GE) | *P. pinaster* | Dead and dying trees, insect attack | *M. g. galloprovincialis* | 2 |
| 17/3 | Friuli V.G. | Sagrado (GO) | *P. sylvestris* | Dead tree, blue stain | | |
| 18/2 | Friuli V.G. | S. Pier d'Isonzo (GO) | *P. sylvestris* | Partially damaged | | |
| 19/2 | Friuli V.G. | S. Pier d'Isonzo (GO) | *P. nigra austriaca* | Dying trees | | |
| 20/4 | Friuli V.G. | Monfalcone (GO) | *P. nigra austriaca* | Partially damaged, resinous exudation, reddish needles | | |
| 21/2 | Friuli V.G. | Monfalcone (GO) | *P. nigra austriaca* | Partially damaged, resinous exudation, reddish needles | | |
| 22/1 | Friuli V.G. | Monfalcone (GO) | *P. halepensis* | Partially damaged, resinous exudation, reddish needles | | |
| 23/4 | Friuli V.G. | Ca' Marin (GO) | *P. halepensis* | Dead trees | | |
| 24/4 | Friuli V.G. | Duino (TS) | *P. nigra austriaca* | Dead trees | | |
| 25/2 | Friuli V.G. | Medeazza (TS) | *P. nigra austriaca* | Dead trees | | |
| 26/4 | Friuli V.G. | Monte Slivia (TS) | *P. nigra austriaca* | Dead trees | | |
| 27/2 | Friuli V.G. | Monte Balico (TS) | *P. nigra austriaca* | Dead trees | | |
| 28/1 | Friuli V.G. | Monte Balico (TS) | *P. halepensis* | Dead trees, insect attack | *M. galloprovincialis pistor* | 3 |
| 29/3 | Friuli V.G. | Duino (TS) | *P. nigra austriaca* | Dying trees | | |
| 30/2 | Friuli V.G. | Monte Deslio (TS) | *P. nigra austriaca* | Dead trees | | |
| 59/1 | Friuli V.G. | Duino (TS) | *P. nigra austriaca* | Insect attack | *Rhagium inquisitor* | 8 |
| 57/1 | Friuli V.G. | Arta Terme (UD) | *P. sylvestris* | Insect attack, reddish needles | *M. g. pistor*<br>*Acanthocinus aedilis* | 2<br>1 |
| 31/3 | Veneto | Ospitale (BL) | *P. sylvestris* | Dead and dying trees, dying shoot | | |
| 32/1 | Veneto | Auronzo (BL) | *P. sylvestris* | Dying shoot | | |
| 33/2 | Veneto | Passo Mauria (BL) | *P. sylvestris* | Dying shoot, fallen trees | | |
| 34/2 | T. Alto Adige | Laces (BZ) | *P. sylvestris* | Dying shoot | | |
| 35/2 | T. Alto Adige | Laces (BZ) | *P. sylvestris* | Dying shoot | | |
| 36/1 | T. Alto Adige | Lacinigo (BZ) | *P. sylvestris* | Dying shoot | | |
| 37/2 | T. Alto Adige | Schulmes (BZ) | *P. sylvestris* | Dying shoot | | |
| 38/2 | T. Alto Adige | Nanturno (BZ) | *P. sylvestris* | Dead trees, blue stain | | |
| 39/2 | T. Alto Adige | Nanturno (BZ) | *P. sylvestris* | Dead trees, dying shoot | | |
| 40/1 | Tuscany | Larciano (PT) | *P. pinaster* | Dying shoot | | |
| 41/4 | Tuscany | Larciano (PT) | *P. pinaster* | Dying shoot | | |
| 42/3 | Tuscany | Montefalcone (PT) | *P. pinaster* | Reddish needles | | |
| 43/3 | Tuscany | Montefalcone (PT) | *P. pinaster* | Reddish needles, fallen tree | | |
| 44/2 | Tuscany | Pescia (PT) | *P. pinaster* | Reddish needles | | |
| 45/1 | Tuscany | Marina di Massa (MS) | *P. pinaster* | Fallen trees | | |
| 46/2 | Tuscany | Marina di Massa (MS) | *P. pinea* | Reddish needles | | |
| 47/2 | Tuscany | Marina di Massa (MS) | *P. pinaster* | Insect attack | *Horthotomicus erosus* | 30 |
| 48/1 | Tuscany | Marina di Massa (MS) | *P. pinea* | Reddish needles | | |
| 49/2 | Tuscany | Marina di Massa (MS) | *Juniperus* sp. | Stem wounds | | |
| 50/3 | Tuscany | Marina di Massa (MS) | *P. pinaster* | Resinous exudation | | |
| 51/1 | Tuscany | Marina di Massa (MS) | *P. pinaster* | Fallen tree | | |
| 52/3 | Tuscany | Piombino (LI) | *P. halepensis* | Insect attack | Bark beetles | 6 |
| 53/1 | Tuscany | Piombino (LI) | *P. pinea* | Insect attack, Blue stain | Flatheaded wood borers<br>*Azhopalus* sp. | 7<br>4 |
| 54/1 | Tuscany | Piombino (LI) | *P. pinaster* | Stem wounds | | |
| 55/3 | Tuscany | Piombino (LI) | *P. halepensis* | Resinous exudation | | |

TABLE I - *Continued.*

| Sample No* | Region | Locality (Province) | Tree species | Condition of trees** | Insect captured | |
|---|---|---|---|---|---|---|
| | | | | | Species | No |
| 56/4 | Tuscany | Piombino (LI) | *P. halepensis* | Insect attack | *Tomicus destruens* | 12 |
| | | | | | *Horthotomicus erosus* | 15 |
| 58/3 | Tuscany | Scopeti (FI) | *P. pinea* | Blue stain, insect holes | | |
| 7/6 | Piedmont | Ovada (AL) | *P. pinaster* | Partially damaged, insect attack, dying shoot | *Monochamus* (larvae) | 7 |
| | | | | | *Arhopalus rusticus* | 2 |
| 8/2 | Piedmont | Ovada (AL) | *P. nigra austriaca* | Dying shoot | | |
| 9/1 | Piedmont | Ovada (AL) | *P. pinaster* | Dead tree | | |
| 60/4 | Piedmont | Fenestrelle (TO) | *P. sylvestris* | Dying shoot | | |
| 61/3 | Piedmont | Oulx (TO) | *P. sylvestris* | Insect attack | *Pissodes* sp. | 12 |
| 62/5 | Piedmont | Variselle (TO) | *P. nigra austriaca* | Reddish needles | | |
| 63/2 | Piedmont | Dronero (CN) | *P. strobus* | Shoot dying | | |
| 64/1 | Piedmont | Monte Calvario (CN) | *P. sylvestris* | Reddish needles, dying shoot | | |
| 65/3 | Piedmont | S. Anna (CN) | *P. sylvestris* | Insect attack | *Pissodes* sp. | 15 |
| 66/3 | Piedmont | Ovada (AL) | *P. pinaster* | Dying shoot | | |
| 67/2 | Piedmont | Castello (AL) | *P. pinaster* | Reddish needles | | |
| 68/2 | Piedmont | Mond'Ovile (AL) | *P. pinaster* | Reddish needles, dying shoot | | |

* Progressive number/total number of sampled trees.
** Condition of trees is referred to a protocol agreed with the Project-coordinator (FAIR, CT 95-0083).

48 hours, with a 10 ml suspension collected at 24 hours intervals. *Bursaphelenchus* spp. were identified using a stereomicroscope. Some of the specimens of the *Bursaphelenchus* species extracted were fixed in FP 4:1 hot solution, processed by the glycerol-ethanol method (Seinhorst, 1959) and mounted in anhydrous glycerin for specific identification. The remaining specimens were cultured in Petri dishes on *Botrytis cinerea* Pers. at 24 °C. Rearing of *Bursaphelenchus* spp., except *B. mucronatus*, also was attempted on *Monilinia fructicola* (Wint.) Honey, *Monilia* sp., *Beauveria bassiana* (Bals.) Vuill., *Botrytis elliptica* (Berk.) Cooke, *Ceratocystis fimbriata* Ellis *et* Halst, *Seiridium cardinale* (Wagener) Sutton *et* Gibson and *Diplodia pinea* (Desm.) Kicky. *Diplodia pinea* was isolated from pine wood with blue stain.

Each population was labelled as *Bursaphelenchus* (name of species) IT (for Italy) No (progressive number) and w (for wood).

In a search for possible insect vectors, log portions showing insect holes were mantained at room temperature in rearing cages until adult emergence. Emerged insects were identified, dissected and incubated 48 hours in a moist chamber for recovery of nematodes.

## Results

All localities surveyed, coniferous species sampled, their phytosanitary status and insect species found are reported in Table I.

Populations identified as *Bursaphelenchus* species occurred in only three regions, namely Piedmont, Friuli Venezia Giulia and Tuscany (Fig. 1). Species of pine wood nematodes detected, host and isolate numbers are indicated in Table II.

In the survey *B. xylophilus* was never detected. *Bursaphelenchus* species were frequently associated with *Pinus pinaster* Ait. and *P. sylvestris* L. and specimens of *Bursaphelenchus* species were also found in association with *P. halepensis* Mill., *P. pinea L.*, *P. nigra austriaca* Arn. and *P. strobus* L., but never in the wood of *Larix decidua* Mill. and *Juniperus communis* L.

*B. mucronatus* Mamya *et* Enda was the most common species occurring in seven localities in Piedmont and one in Tuscany.

*B. leoni* Baujard was found in six localities in Tuscany and one in Friuli Venezia Giulia.

In Tuscany *B. sexdentati* Rühm was found in five localities and *B. teratospicularis* Kakulia *et* Devdariani in three. Specimens recovered from

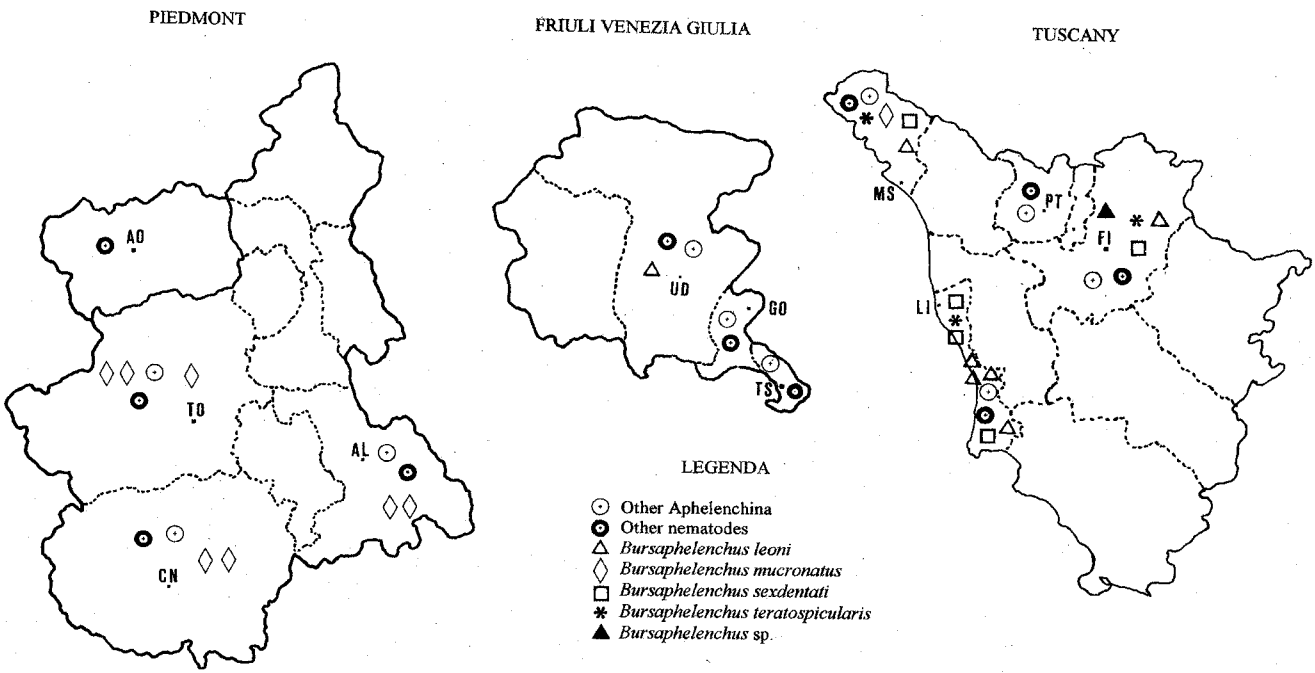


Fig. 1 - Geographical distribution of *Bursaphelenchus* spp. and other nematodes in three Italian regions.

wood of *P. pinea*, at Scopeti, in Tuscany, are considered to represent an undescribed species.

The number of recovered specimens of *B. mucronatus* varied from 1 to 7.6/10 g of dry wood with the highest number in *P. sylvestris* wood. Of the five species of pine wood nematodes identified, *B. sexdentati* had the highest population density, 37.1 specimens/10 g of dry wood of *P. halepensis*.

Mixed populations, two to four species, occurred in four samples collected in Tuscany with the constant presence of *B. leoni* and *B. sexdentati*.

Rhabditida and other Aphelenchida, mainly specimens of *Aphelenchoides* and *Laimaphelenchus*, occurred in most samples. The presence of Tylenchida was occasional and restricted to a few specimens.

*B. mucronatus*, *B. sexdentati* and *Bursaphelenchus* sp. were successfully grown *in vitro* on *B. cinerea* mycelium; the other fungi tested did not support any nematode growth despite several attempts, with the exception of *D. pinea* on which reproduction of *B. sexdentati* occurred. No suitable culture medium was found for *B. leoni* and *B. teratospicularis*.

A total of 132 insect specimens emerged from the wood pieces in rearing cages. They were mainly Scolitydae identified as *Tomicus destruens* (Woll.), *Orthotomicus erosus* (Woll.), followed, in order of frequency, by the Cerambycid *Monochamus g. galloprovincialis* (Ol.), *M. g. pistor* (Germar), *Rhagium inquisitor* (L.), *Acanthocinus aedilis* (L.) and *Arhopalus rusticus* (L.), the Curculionid *Pissodes castaneus* (Degeer) = *P. notatus* (F.), the Buprestidae and the Hymenoptera Siricidae *Sirex juvencus* (L.). Only saprozoic nematodes of the order Rhabditida emerged from the incubated insects.

## Discussion and conclusions

In the Italian coniferous forests generally there are no large areas of declining plants. Symptoms of decline were frequently observed

TABLE II - *Species of pine wood nematodes detected and their hosts.*

| Sample No. | Host | Sample weight g | Dry weight g | *Bursaphelenchus* spp. | Isolate No. | No. of recovered specimens/10 g dry weight |
|---|---|---|---|---|---|---|
| 47/2 | *P. pinaster* | 60 | 38.2 | *B. leoni* | IT1 (w) | 0.5 |
| | | | | *B. sexdentati* | IT2 (w) | 1.6 |
| | | | | *B. teratospicularis* | IT3 (w) | 0.5 |
| | | | | *B. mucronatus* | IT13 (w)* | 1.0 |
| 52/3 | *P. halepensis* | 90 | 47.9 | *B. leoni* | | 0.8 |
| 53/1 | *P. pinea* | 30 | 14.8 | *B. leoni* | | 1.4 |
| 54/1 | *P. pinaster* | 30 | 17.8 | *B. leoni* | | 1.7 |
| | | | | *B. sexdentati* | | 1.1 |
| 55/3 | *P. halepensis* | 90 | 50.5 | *B. sexdentati* | | 2.0 |
| 56/4 | *P. halepensis* | 120 | 49.8 | *B. leoni* | IT10 (w) | 2.0 |
| | | | | *B. sexdentati* | IT9 (w) | 37.1 |
| | | | | *B. teratospicularis* | IT11 (w) | 3.0 |
| 57/1 | *P. sylvestris* | 30 | 18.3 | *B. leoni* | | 1.6 |
| 58/3 | *P. pinea* | 90 | 55.3 | *B. leoni* | | 0.4 |
| | | | | *B. sexdentati* | | 0.4 |
| | | | | *B. teratospicularis* | | 0.2 |
| | | | | *Bursaphelenchus* sp. | IT14 (w) | 0.7 |
| 60/4 | *P. sylvestris* | 120 | 78.4 | *B. mucronatus* | IT4 (w) | 3.2 |
| 61/3 | *P. sylvestris* | 90 | 42.8 | *B. mucronatus* | IT5 (w) | 3.5 |
| 62/5 | *P. nigra austriaca* | 150 | 72.0 | *B. mucronatus* | IT6 (w) | 4.4 |
| 63/2 | *P. strobus* | 60 | 31.8 | *B. mucronatus* | IT7 (w) | 3.8 |
| 64/1 | *P. sylvestris* | 30 | 17.2 | *B. mucronatus* | IT8 (w) | 7.6 |
| 66/3 | *P. pinaster* | 90 | 61.4 | *B. mucronatus* | | 1.0 |
| 68/2 | *P. pinaster* | 60 | 32.4 | *B. mucronatus* | | 3.1 |

* Collected by Dr. H. Braasch (B.B.A., Kleinmachnow, Germany).

in Friuli Venezia Giulia on *P. nigra austriaca*; but they did not appear to be related to the presence of *Bursaphelenchus* populations. The most severe symptoms were observed in Liguria and in some neighbouring areas of Piedmont. In Liguria from *P. pinaster* trees and from specimens of *M. g. galloprovincialis* found in their wood, populations of *B. mucronatus* were extracted during previous surveys (Marinari Palmisano *et al.*, 1992, 1994). However, the scale insect *Matsucoccus feytaudi* Ducasse was considered to be the main cause of such symptoms (Covassi and Binazzi, 1992). Possibly the presence of the scale insect increased plant susceptibility to *B. mucronatus* and *M. g. galloprovincialis*.

In Piedmont, *B. mucronatus* was detected in several cases; however, populations were low and not considered to be harmful to *P. pinaster*.

Generally, possible insect vectors of the pine wood nematode occurred in too few numbers to establish reliable association although in some cases insect populations were enough numerous to be eventually responsible of an epidemics of the nematode.

During the survey *B. mucronatus* was not found in Liguria where it had previously been recorded (Marinari Palmisano *et al.*, 1992).

*B. leoni*, previously reported from France (Baujard, 1980), Italy (Marinari Palmisano and Ambrogioni, 1994; Ambrogioni *et al.*, 1994) and Cyprus (Philis and Braasch, 1996), appeared to be the most widespread species in Italy, although often in low population densities and mixed with other *Bursaphelenchus* species. However, the presence of this species, which causes wilting of *P. brutia* Tenore in Cyprus (Philis, 1996) might represent a threat.

*B. mucronatus* was found in the wood of four pine species, namely *P. pinaster*, *P. sylvestris*, *P. nigra austriaca* and *P. strobus*, the latter three species constituting the first record of association for Italy.

Presence of blue stain fungi was frequently observed in the collected wood. A strain of *D. pinea* was successfully tested as growing medium for *B. sexdentati*.

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