

and on the characteristics of the stands where the traps are being placed; being the latter a key factor, since in the same year in some stands a large number of insects are collected whereas other stands do not record any. However, the factors influencing *M. galloprovincialis* to choose one stand or another are not known, thus insects have been captured in apparently healthy stands and without any other favourable required conditions (wood processing facilities nearby...).

In our region, adult captures begin in May or June depending on the year, with a peak in July or August and descending from that time onwards. Insects are normally captured until September, later *M. galloprovincialis* specimens are only recorded in some traps and in isolated cases. *M. sutor*, the other species reported in Spain has been rarely detected, only during Summer, and in a low number.

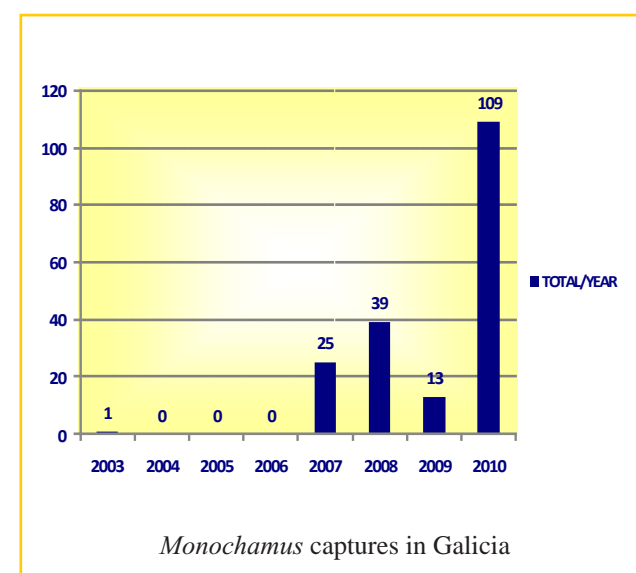
In general, the insect has been more commonly found in the southern Galician provinces, but in some years the largest number of specimens was recorded in A Coruña province. In 2010, when *B. xylophilus* was detected in As Neves, the largest number of *Monochamus galloprovincialis* in Galicia was recorded in this municipality. This year also registered the largest number of captures as compared to the rest of years (even the global figures recorded this year have been higher than the sum of the captures registered in previous years).

So far *B. xylophilus* has not been yet detected in any of the *M. galloprovincialis* specimens collected so far in Galicia.

Control

Once the nematode is detected in the tree it has proved impossible to control the nematode, but since it is regarded as a quarantine organism, the government will carry out the adequate measures to eradicate the pathogen.

However, the best option to prevent disease spread is to place traps in areas where the insect is known to occur in order to contain insect populations and to carry out silvicultural measures. Traps will be placed before adult activity is initiated (May); silvicultural measures will be aimed to keep pine woods in good condition, by removing death trees, piles of wood from felled trees...



Multi-funnel trap to capture the insect vector

Current situation of *Bursaphelenchus xylophilus* in Galicia

Bursaphelenchus xylophilus (Steiner et Buhrer, 1934) Nickle, 1970, agent of conifer wilt and decay, is regarded a quarantine organism according to Council Directive 77/93/EEC on protective measures against the introduction into the Member States and spread of harmful organisms of plants or plant products. In 2006 the decision 2006/133/EC which requires Member States temporarily to take measures against the dissemination and introduction of *B. xylophilus* in their countries is published.

Similarly, the EPPO (European and Mediterranean Plant Protection Organization) includes *B. xylophilus* in A2 list, which comprises those pests that are locally present in the EPPO region.

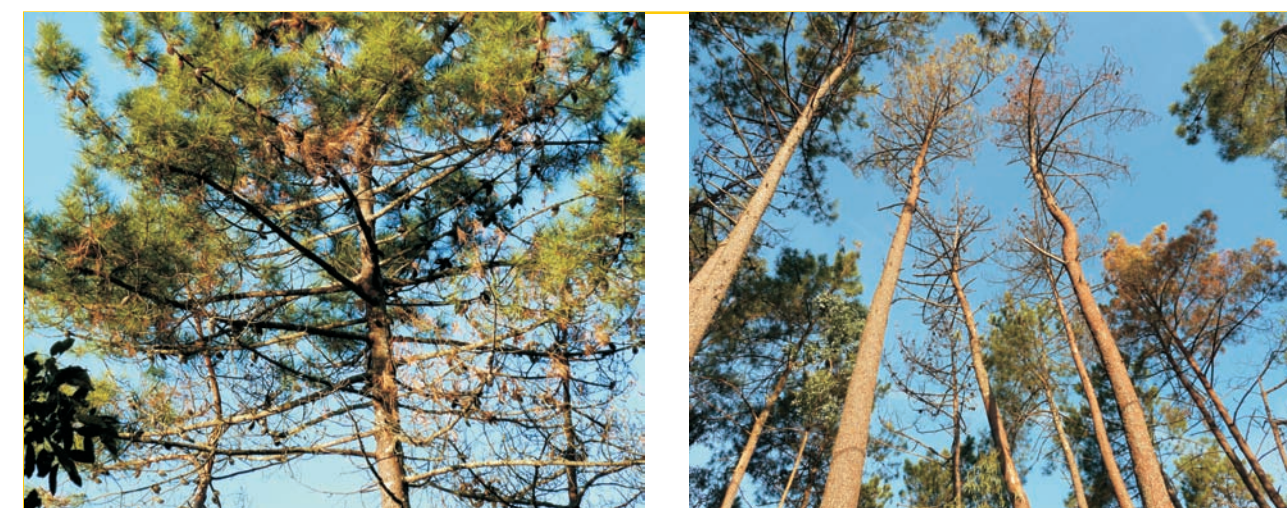
Bursaphelenchus xylophilus has been first reported in Europe in 1999 in Portugal. Since then, extensive surveys in conifer stands, sawmills and other wood processing companies have been carried out in all European countries, and mainly in Spain, with the aim to determine the potential occurrence of this quarantine pathogen.

In Galicia (NW Spain) during this period, 5155 samples have been analyzed following the sampling guidelines established by the standards and directives that have been in force during the surveillance period.

Analysis of these samples showed that ten species belonging to genus *Bursaphelenchus* have been found, namely *B. mucronatus* Mamiya et Enda, *B. sexdentati* Rühm, *B. eggersi* Rühm, *B. hylobianum*



B. xylophilus adult specimen



Symptoms caused by *B. xylophilus* in *Pinus pinaster*

(Korenchenko) Hunt, *B. pinasteri*, Baujard, *B. leoni* Baujard, *B. teratospicularis* Kakuliya et Devdariani *B. tusciae* Ambrogioni & Marinari-Palmisano, *B. antoniae* Penas, Metge, Mota & Valadas and *B. hildegardae* Braasch, Burgermeister, Schönfeld, Metge & Brandstetter, being the latter three reported for the first time in Spain.

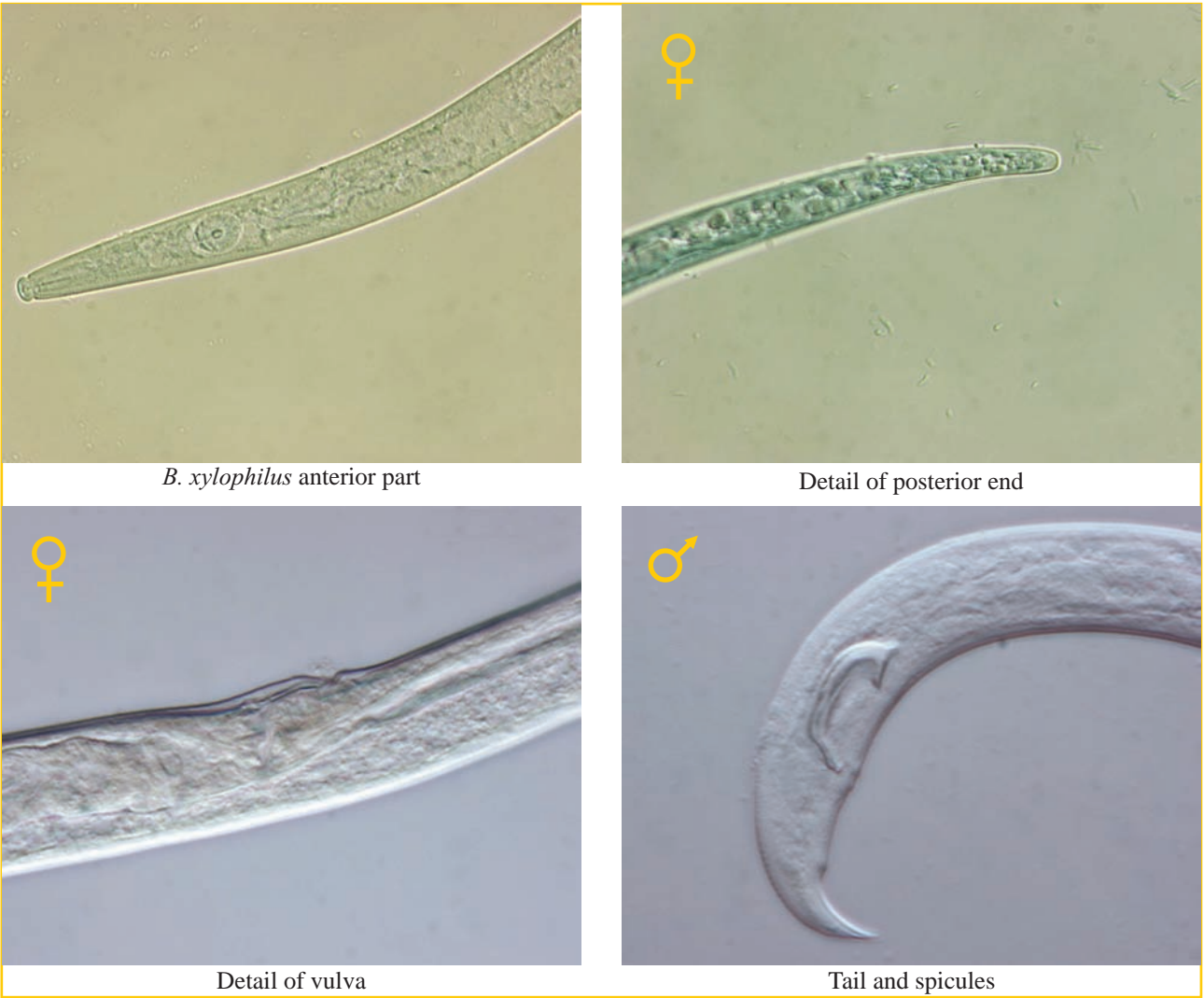
After the declaration of continental Portugal in 2008 as demarcated area, these surveys have been increased in Spain, mainly along the border with Portugal. In the same year in Spain, a tree affected by *B. xylophilus* was detected in Villanueva de la Sierra, Cáceres.

Bursaphelenchus xylophilus was first detected in Galicia during the extensive surveys carried out in 2010. The nematode was found 20 Km away from the Portuguese border in a *Pinus pinaster* stand in As Neves, Pontevedra province, in a 2 x 2 Km grid pattern.

Nematode identification

Once in the laboratory the samples collected in the surveys (trunk shavings) are incubated for 15 days at 25°C; then they are processed according to the Baermann funnel technique. The occurrence and the number of nematodes are determined under a magnifying glass.

The identification of the genus and species found in the samples was carried out by morphological, morphometric and molecular techniques.



Regarding *B. xylophilus* morphology, this species shows three characters different from the rest of *Bursaphelenchus* species:

- 1. In the female the anterior vulvar lip is a distinct overlapping flap.
- 2. The posterior end of female body is rounded in nearly all individuals.
- 3. In the males the spicule is flattened into a disc-like structure named *cucullus*.

B. xylophilus identification by molecular techniques was performed following three different protocols, all of them recommended by the EPPO. They were the following: 1) PCR amplification of *Bursaphelenchus xylophilus* satellite DNA obtaining the fragments of 160bp, 320bp, 480bp characteristic of the species. 2) Amplification by real-time PCR using a Taqman probe in a 77bp region of *Bursaphelenchus xylophilus* satellite DNA. 3) PCR-RFLP of the rDNA ITS region of *Bursaphelenchus xylophilus* obtaining the restriction pattern of *B. xylophilus*. Sequencing of the PCR product showed a 99% homology with *B. xylophilus* sequences of the ITS region deposited in the GenBank.

Monochamus galloprovincialis* (Olivier, 1795) vector of *Bursaphelenchus xylophilus

Since the nematode was first reported in Portugal, extensive surveys have been carried out in Galicia to determine the occurrence and distribution of its main vector, the cerambycid *Monochamus galloprovincialis*. This insect is surveyed using multi-funnel traps with aggregation pheromones of *Scolytidae* (*M. galloprovincialis* adults are attracted to these traps) or with recently developed specific pheromones. In addition, pine volatile compounds are also added, in our case, alpha-pineno.

Traps are placed every year in the whole Galician territory, but the largest number of traps was installed along the Portuguese border (southern municipalities in Pontevedra and Ourense provinces), mainly in *Pinus pinaster* stands (some traps have been also placed in *P. radiata*). They are usually installed in May-June and are maintained until September-October or even later in case insects are also captured during late autumn and winter seasons.

The first *Monochamus galloprovincialis* adult was captured in a trap in Ourense province in 2003; on the following three years no new captures were recorded; but since 2007 insects are being collected in the traps, but differing on the number of insects collected depending on the environmental conditions

