Pine Wilt Disease: A Worldwide Threat to Forest Ecosystems

Manuel M. Mota · Paulo Vieira
Editors

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Preface

Pine wilt disease (PWD) is unquestionably a major threat to forest ecosystems worldwide. After seriously affecting Eastern Asian countries, the challenge is now in Europe, following its detection in Portugal in 1999 and its subsequent spread.

For foresters, these were really very bad news and, in order for adequate action to be taken, scientists had to teach politicians about the seriousness of the problem. That is never an easy task, but it was successfully done at that time, mainly by the continued effort of Professor Manuel Mota.

The challenge of having political decisions based on good science is fundamental for the success of any program, but especially in difficult situations such as those arising by the introduction of harmful organisms in new ecosystems. The success of the dialogue between science and policy requires intelligent partners from each side, which is not always necessarily the case. . .

Examples of lack of recognition of problems raised by science are unfortunately abundant throughout the history of science. The recent recognition of the efforts of the Intergovernmental Panel on Climate Change (IPCC) and Al Gore with the Nobel Prize, and the continued failure in taking appropriate actions by major political players is a dramatic modern example of the difficulty of this dialogue. . .

These are some of the reasons why I think that this book plays a fundamental role in the issue of pine wilt disease: Firstly, the book addresses a very important problem that threatens the ecological and economical balance of many forested areas worldwide. Secondly, it assembles contributions of the best specialists worldwide in the various facets of the problem. Thirdly, it summarises knowledge in an attempt to make it useful for adequate action. Finally, it provides insights for future developments in scientific research.

I had already the privilege of addressing some words of recognition to the participants of the PWD Conference at the Gulbenkian Foundation in Lisbon in July 2006 where I was very much impressed with the importance and the quality of the contributions. As Director of the Portuguese Forest Services (DGRF) at that time and until November 2007, I must stress that this Conference was very instrumental in setting the stage for discussions and for the planning of new strategies in dealing with the issue of the presence of the pinewood nematode in Portugal.

For these new strategies important scientific contributions were given by Edmundo Sousa (another relevant participant to the Conference) in addressing the issues related to the spread of the insect vector.

I would like to take this opportunity to stress my recognition for the tremendous and unique work done by the team of the Forest Services, coordinated by José Manuel Rodrigues (a contributor to this book) that resulted in the establishment in early 2007 of a clearcut belt 430 km long and 3 km wide around the affected zone. This strategy was adopted and financially supported by the European Commission, which sent several missions to Portugal. The mission leaders, in November 2007, expressed satisfaction with the success of this extremely difficult operation.

We do not know, at this moment, what will be the final effectiveness of this new strategy. We do know, however, that without this major effort the hope of success for the eradication program would be minimal. I am sure that, until November 2007, the Forest Services did everything it was possible, by dedicating human and financial resources, by taking the necessary risks and facing lack of understanding, to ensure that appropriate action was taken, making use of the best science available. And I hope that this effort will be continued with the same strength in the future. . .

I am certain that this book, absolutely necessary for those who want to act in a responsible manner in the very difficult combat against the spread of pine wilt disease, constitutes also a fundamental contribution for the advancement of science and for the stimulus of future research in this field.

For the courageous editors and for the excellent contributors to the conference and the book, I would like to reiterate my sincere recognition and gratitude, that I am sure will be shared by all of those who care for forests around the world.

Thank you!

Lisbon
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Francisco Castro Rego

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Part I
Pine Wilt Disease: Global Issues, Trade and Economic Impact

John Webster and Manuel Mota

Summary

Pine wilt disease (PWD) is perhaps the most serious threat to pine forests worldwide. Since it's discovery in the early XXth century by Japanese forest researchers, and the relationship with its causative agent, the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, in the 1970s, PWD has wreaked havoc wherever it appears. Firstly, in the Far East (Japan, China and Korea) and now, more recently in 1999, in the EU (Portugal).

The forest sector in Portugal plays a major role in the Portuguese economy with a 12% contribution to the industrial gross domestic product, 3.2% of the gross domestic product, 10% of foreign trade and 5% of national employment. Maritime pine (*Pinus pinaster*) is one of the most important pine productions, and industrial activity, such as the production of wood and resin, as well as coastal protection associated with sand dunes. Also, stone pine (*Pinus pinea*) plays an important role in the economy with a share derived from the exports of high-quality pineon seed. Thus, the tremendous economical and ecological impact of the introduction of a pest and pathogen such as the PWN, although as far as is known, the only species susceptible to the nematode is maritime pine.

Immediately following detection, the research team involved (Univ. Évora, INIAP) informed the national plant quarantine and forest authorities, which relayed the information to Brussels and the appropriate EU authorities. A task force (GANP), followed by a national program (PROLUNP) was established. Since then, national surveys have been taking place, involving MADRP (Ministry of Agriculture), the University of Évora and several private corporations (e.g. UNAC). Forest growers in the area are particularly interested and involved since the area owned by the growers organizations totals 700 000 ha, and is largely affected by PWD. Detection of the disease has led to serious consequences and restrictions regarding exploration and commercialization of wood. A precautionary phytosanitary strip, 3 km wide, has been recently (2007) established surrounding the affected area. The Portuguese government, through its national program PROLUNP, has been deeply involved since 1999, and in conjunction with the EU (Permanent Phytosanitary Committee, and FVAO) and committed to controlling this nematode and the potential spread to the rest of the country and to the rest of the EU.

The global impact of the presence of *Bursaphelenchus xylophilus* or the threat of its introduction and the resulting pine wilt disease in forested areas in different parts of the world is of increasing concern economically. The concern is exacerbated by the prevailing debate on climate change and the putative impact this could have on the vulnerability of the world's pine forests to this disease. The scientific and regulatory approach taken in different jurisdictions to the threat of pine wilt disease varies from country to country depending on the perceived vulnerability of their pine forests to the disease and/or to the economic cost due to lost trade in wood products.

Much of the research surrounding pine wilt disease has been located in the northern hemisphere, especially in southern Europe and in the warmer, coastal, Asian countries. However, there is an increased focus on this problem also in those countries in the southern hemisphere where plantations of susceptible pine have been established over the years. The forestry sector in Australia and New Zealand are on "high alert" for this disease and are practicing strict quarantine procedures at all ports of entry for wood products. As well, there is heightened awareness, as there is worldwide, for the need to monitor wood packaging materials for all imported goods.

In carrying out the necessary monitoring and assessment of products for *B. xylophilus* and its vectors substantial costs are incurred especially when decisions have to be made rapidly and regardless of whether the outcome is positive or negative. Australia's response recently to the appearance of some dying pines in a plantation illustrated the high sensitivity of some countries to this disease. Some $200 000 was spent on the assessment in order to save a potential loss of millions of dollars to the disease. This rapid, co-ordinated response to the report was for naught, because once identified it was found not to be *B. xylophilus*. This illustrates the particular importance of taking the responsibility at all levels of management to secure the site and the need of a rapid, reliable diagnostic method for small nematode samples for use in the field.

Australia is particularly concerned about the vulnerability of its 1million hectares of planted forests, 80% of which are *Pinus* species, to attack from incursions of one or more species of the insect vector. *Monochamus alternatus* incursions in wood pallets have been reported from Brisbane, Queensland. The climate of this part of Australia is such that the *Pinus* plantations are particularly vulnerable to the potential outcome of such incursions, and the state of Queensland is developing a risk management strategy and a proactive breeding programme in response to this putative threat.

New Zealand has 1.6 million hectares of planted forests, and 89% of the commercial forest is *Pinus radiata*. Although the climate where these forests are located tends to be somewhat cooler than that in Australia the potential for establishment and development of the disease in that country is believed to be high. The passage alone of 200 000 m^3/year of wood packaging through New Zealand ports is itself sufficient to require response. The potential incursion of insect vectors of pinewood nematode through the port system is regarded as high and is monitored carefully.

The enormous expansion of global trade and the continued use of unprocessed/inadequately-processed wood for packaging purposes is a challenge for all trading nations as such wood packaging material often harbours disease or pest species. The extent of this problem is readily illustrated by the expanding economies and exports of countries in south-east Asia, China, Japan and Korea have significant areas of forestland infested with *B. xylophilus*. These countries too are among the largest exporting countries of manufactured goods. Despite the attempts of authorities to ensure that only properly treated wood is used in the crating and packaging of goods *B. xylophilus* and/or its insect vector infested materials is being recorded at ports worldwide. This reminds us, therefore, of the ease with which this nematode pest can gain access to forest lands in new geographic locations through inappropriate use, treatment or monitoring of wood products. It especially highlights the necessity to find an alternative to using low-grade lumber for packaging purposes.

Lest we should believe that all wood products are always carriers of *B. xylophilus* and its vectors, it should be remembered that international trade of all kinds has occurred for thousands of years and that lumber-born pests and diseases do not have worldwide distribution. Other physico-biological factors have a significant role in the occurrence, establishment and sustainability of a disease. The question is often raised as to why the whole of southern Europe doesn't already have *B. xylophilus* and pine wilt disease. European countries have traded with countries that are infested with *B. xylophilus* for hundreds of years. Turkey is an example of a country that appears to be highly vulnerable to pine wilt disease due to its extensive forests in the warm, southern region where the vector, *Monochamus galloprovincialis*, occurs. However, there is no record of the presence of *B. xylophilus* occurring there despite the importation of substantial quantities of wood from several countries

In many respects, Portugal illustrates both the challenge and the dilemma. In recent times *B. xylophilus* was discovered there in the warm coastal region. The research, administrative and quarantine authorities responded rapidly and *B. xylophilus* appears to have been confined to the region in which it was found. The rapid response would seem to have "saved the day" for Portugal. Nevertheless, it raises again the long-standing questions, how long had *B. xylophilus* been in Portugal before it was found? If Lisbon was the port of entry, which seems very likely, why had *B. xylophilus* not entered Lisbon many years earlier and established populations and the pine wilt disease? Will the infestation in Portugal be sustainable and will it spread or will it die out within a few years? We still do not have sufficient understanding of the biology of this pest to know the answers to these questions.

National Eradication Programme for the Pinewood Nematode

José M. Rodrigues

Introduction

The pinewood nematode (PWN),[1] *Bursaphelenchus xylophilus*, is listed as a harmful organism to plants or plant products by the European Union (EU) (Annex II, Council Directive 2000/29/EC of 8 May 2000). Its introduction into and spread within all Member States must be banned. This organism, the causal agent of pine wilt disease (PWD), is a serious pest and pathogen of forest tree species, in particular among the genus *Pinus*; its presence in the territory of a member state obliges the country to notify the partners and to adopt immediate safeguard measures. The subjects of contamination are plants of the genus *Abies, Cedrus, Larix, Picea, Pinus, Pseudotsuga*, and *Tsuga*, with the exception of their fruits and seeds, and wood of conifers (Coniferales), originating from non-European countries.

In May 1999, the PWN was detected in Portugal, in dead maritime pine stands located in the Setúbal Peninsula. Following Council Directive 2000/29/EC, the Portuguese authorities informed the European Community and implemented a phytosanitary strategy with the purpose of controlling and eradicating the pest, a programme known as the National Eradication Programme for the Pinewood Nematode (PROLUNP). At the EU level, the situation has been discussed at the Permanent Phytosanitary Committee. Since the pest was recorded in Portugal, several inspection missions have been carried out by the Food and Veterinary Office (DG SANCO). The legal basis for the implementation of this Program is the Executive-Law n.º 154/2005 (Sept. 6th), which establishes the general phytosanitary

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[1] The PWN has had devastating effects on pines forests in East Asian countries, as in Japan, for instance. The nematode is transported as fourth-stage dispersal juveniles by cerambycid beetles of the genus *Monochamus*; in Portugal the PWN was found associated with the species *M. galloprovincialis*, which can attack and infect healthy trees and colonise weakened trees with its offspring.

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rules for Portugal, and Regulation n.º 103/2006 (Feb. 6th) as amended by Regulation n.º 815/2006 (Aug. 16th) and Regulation n.º 321/2007 (March 23rd).

Phytosanitary Strategy

In general, the pursued phytosanitary strategy, delineated to avoid the dispersion of the disease, has been the elimination of decline symptomatic trees,[2] identified through the execution of surveys (during the autumn-winter period), complemented with the control of the insect vector population (during the spring-summer period) and the control of coniferous wood flows (during all year).

Even though PROLUNP covers all mainland Portugal, the fact that the PWN is confined to a certain region, led to the definition of a Demarcated Area (DA), subdivided into an Affected Zone (AZ),[3] a Buffer Zone (BZ)[4] and the remainder of the territory, the Free Zone (FZ)[5] in which risk areas can be found, i.e. places where conifer wood (raw and processed) is stored, and subject of periodic monitorization (Fig. 1). Two critical locations (CL), i.e. clearly delimited areas in which there is a

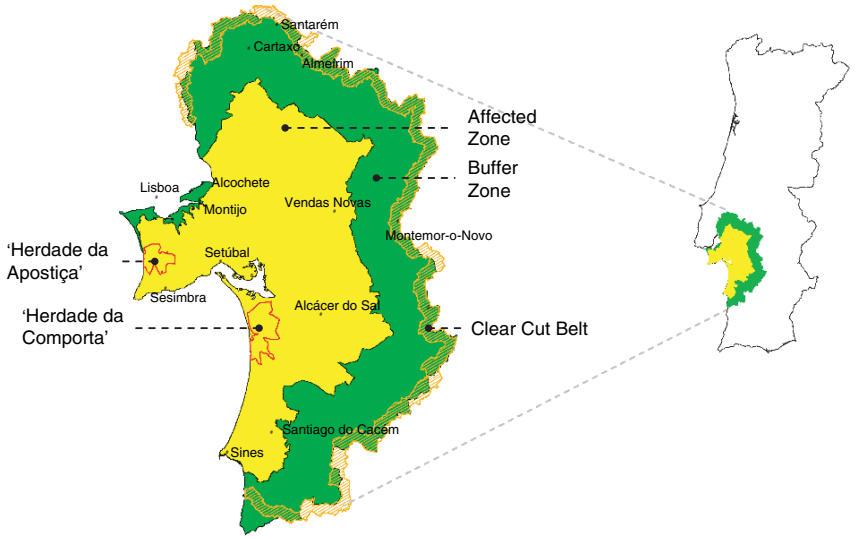

Fig. 1 PROLUNP territorial coverage. The Demarcated Area (Affected Zone and Buffer Zone), Clear Cut Belt and Critical Locations are depicted

[2] Decline symptomatic trees – trees found to be infested by the PWN, showing symptoms of poor health, or located in salvage areas (cf. Annex to Commission Decision 2006/133/EC).

[3] Affected Zone – area in which the pine wood nematode is known to occur.

[4] Buffer Zone – area surrounding the Affected Zone, of no less than 20 km width, where the pine wood nematode is not known to occur.

[5] Free Zone – Area of the territory in which the PWN does not occur.

higher incidence of decline symptomatic trees, are located within the Affected Zone, namely Herdade da Comporta and Herdade da Apostiça.

The Affected Zone covers, currently, 510.000 ha and is surrounded by a Buffer Zone (BZ) of approximately 500.000 ha. The sum of both (1.010.000 ha) constitutes the Demarcated Area (DA), which is subjected to periodic survey, eradication and insect vector control actions and where all forestry activities relating to conifers are subjected to intensive control. The Demarcated Area has changed over the years as result of the evolution of the disease (Fig. 2).

The results of the 2005/2006 surveying and eradication campaign indicated a considerable increase of symptomatic trees throughout the Demarcated Area. This increase was even more evident in the Affected Zone. Several samples collected from the Buffer Zone tested positive for PWN and therefore the Affected Zone and the Demarcated Area limits were redefined. Furthermore, it was decided to create a corridor free from the PWN and its vector host trees, *Picea orientalis, Pinus halepensis, P. nigra, P. nigra laricio, P. pinaster, P. radiata* and *P. sylvestris*, in the periphery of the Demarcated Area, the Clear Cut Belt (CCB), with the purpose of minimizing the possibilities of disease dispersion, as proposed in the 2006 Action

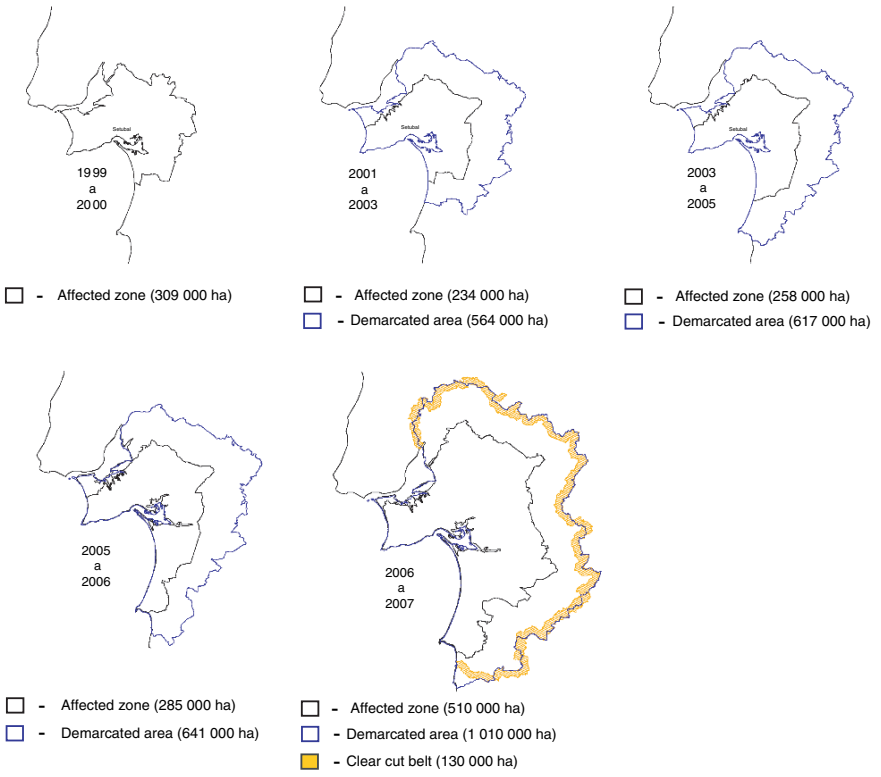

Fig. 2 Evolution of the Demarcated Area and Affected Zone

Plan, presented to the Standing Committee on Plant Health and in accordance with Article 2 of the Commission Decision 2006/133/EC (revised July 2006).

Survey in the Free Zone

The survey carried out in this area aims to monitor conifer forests, focusing in particular on surrounding areas where there is a lot of wood risk materials, whether wood in its natural state or processed wood (risk areas). It also aims to inspect and assess conifers located in permanent plots in each risk area, test all the collected material for PWN in duly accredited laboratories for this purpose and ensure that the methods and procedures provided for in the EU monitoring protocol for PWN are applied correctly.

The survey activities in the Free Zone will also include areas which may be highly attractive for breeding of *Monochamus galloprovincialis*, especially those surrounding the demarcated area, where the survey of coniferied stands was intensify by establishing 200 extra plots. In these plots, samples are taken also from non-symptomatic trees at different heights, including canopy level, and are incubated in order to screen for the presence of PWN.

Survey and Eradication in the Demarcated Area

The aim of the monitoring and the eradication actions within the Demarcated Area is to detect and eliminate all the trees showing symptoms of decline. This area is divided for survey purposes into 136 units of approximately 7 500 ha each. The symptomatic trees are registered within a specific and appropriate geo-referenced matrix, using specific 150 ha maps (Fig. 3) and screening analysis for the PWN presence carried out, in all the identified trees, for the ones located in the Buffer Zone, and in a sample of randomly chosen trees, for the ones located in the Affected Zone. Surveying has been conducted mainly by Forest Owners' Associations, as their knowledge regarding the local sensibilities is valuable. Samples were collected from symptomatic and non-symptomatic trees, taken at different heights, including canopy level, in order to screen for the presence of PWN; some of those samples were incubated (see Chapter 4. for details). Monitoring work is usually expected to start around November and to be concluded in the beginning of February, starting from the periphery to the interior of the Demarcated Area.

The eradication activity consists in the elimination of all the conifers identified by the surveying action guaranteeing the destruction/processing of all the felled trees, according to the Law (Regulation of the Ministry of Agriculture, Rural Development and Fisheries n. º 103/2006, Feb. 6th, as amended by Regulation n. º 815/2006, Aug. 16th, and Regulation n. º 321/2007, March 23rd). The forest owners, farmers or usufructuaries are accountable for the eradication and are informed about the basic lines of action via public notices sent to the local administration, published

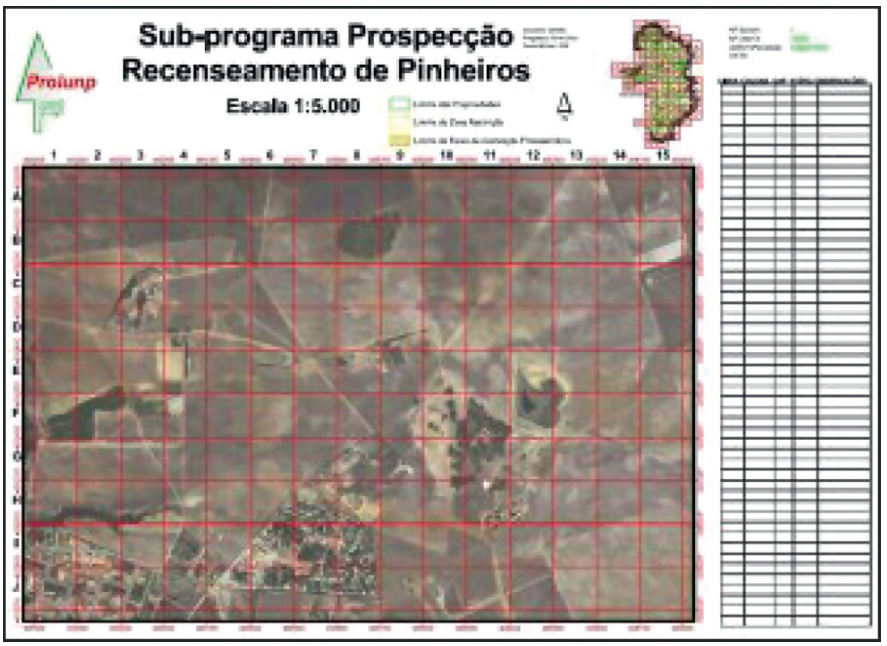

Fig. 3 Example of a surveying 150 ha map

in newspapers and available online; when the owners do not perform the actions themselves, the government must replace them. To do so, it is necessary to sub-contract the eradication services from private companies.

The eradication procedure is expected to guarantee the felling and elimination of all trees identified with decline symptoms, during the period of December 1st to April 1st, the non-flying period of the insect vector's life cycle.

Implementation of a Barrier Free from PWN Vector Hosts (Clear Cut Belt)

The establishment of a Clear Cut Belt intended to set up a corridor (3 km wide) free from PWN vector hosts, roughly following the limits of the most recently defined Demarcated Area, mostly in the Buffer Zone. In this corridor, with an area of about 130 000 ha, all conifers regarded as hosts of *M. galloprovincialis* must be detected, located and eliminated, both declining and healthy ones. To do so, it was necessary to sub-contract the eradication services from private companies.

The Commission Decision 923/2006/CE (Dec. 13th), created a financial contribution for 2006 and 2007 to cover expenditure incurred by Portugal for the purpose of controlling the PWN, considered compensation payment of a compensation for the value of the wood to tree owners or beneficial owners.

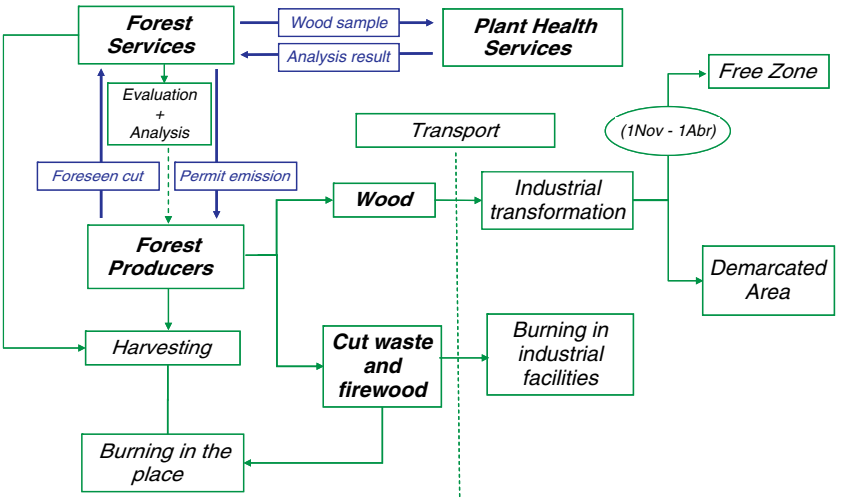

Fig. 4 Wood flow control system

Insect Vector Control

Besides de identification and elimination of decline symptomatic trees, the control of the insect vector populations can be used as an additional strategy to control PWN dispersion. This has been done through the use of a network of traps set along the outer limit of the Affected Zone, which capture the insect during its flight period (spring and summer). Research is in progress regarding the development of a more appropriate and more effective trap.

Inspection and Control of Coniferous Wood

PROLUNP set up a wood trace back system, which compels owners to apply for a conifers' felling and transport permit in the Demarcated Area. After inspection, the phytosanitary inspectors authorize the cuts (permit emission) and the wood destination (Fig. 4), granted that the notification is in accordance with the legal disposaitions. The inspectors also control the authorized destinations in order to assure the fulfillment of phytosanitary measures.

The Disease in Portugal

The number of decline symptomatic trees, potentially infested with PWN, has been increasing since the disease was detected, a trend which is not confirmed by the 2006/2007 data. In what concerns the Buffer Zone, the number of eradicated trees has been generally the same along the years (Fig. 5). However, it is important to note

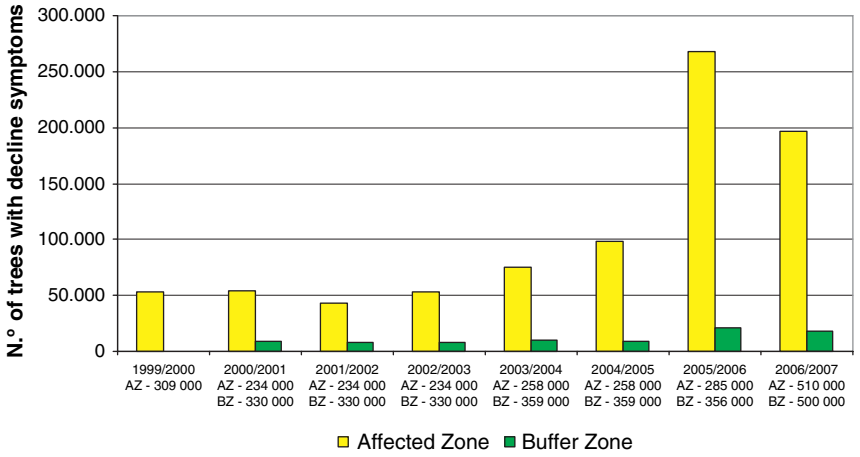

Fig. 5 Decline symptomatic trees evolution in the Demarcated Area. Also depicted is the area covered by the Affected Zone and by the Demarcated Area, in ha

that the Affected Area has been changing along the years and also that the decline symptomatic trees are not necessarily infested with the PWN, research showing that the conifers' decline causal agents, in the region, are rather diverse, biotic and abiotic. This can be deduced by the analysis of the graphic presented on Fig. 6 that shows a distinct trend in both indexes [Number of decline symptom trees/DA pine stand area] and [Estimated number of positive trees/Number of decline symptomatic trees], along the different campaigns. This suggests that other decline causal agents, rather than PWN, are present and might be responsible for the decline increase.

In the Affected Zone, decline symptomatic trees are concentrated in some important production areas, the "critical locations". Table 1 indicates the evolution of total number of decline symptomatic trees identified in the Affected Zone, as well

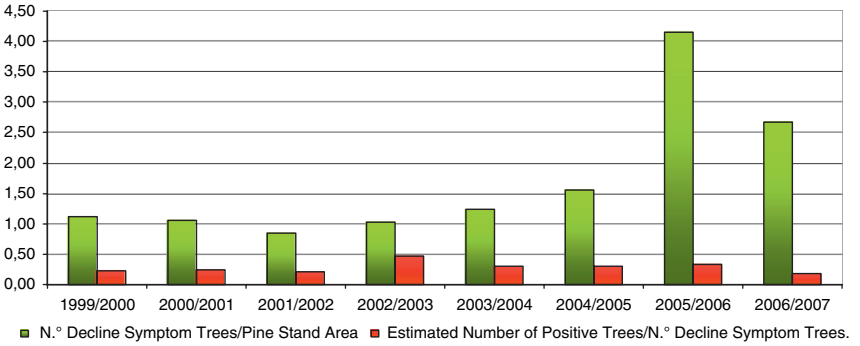

Fig. 6 Evolution of the indexes [Decline symptomatic trees/DA Pine stand area] and [Estimated number of positive trees/Decline symptomatic trees]

Table 1 Evolution of the number of decline symptomatic trees in the Affected Zone and in the Critical Locations, Comporta e Apostiça. For reference the estimated number of maritime pines, in the Affected Zone, is 7 millions

CAMPAIGN	99/00	00/01	01/02	02/03	03/04	04/05	05/06	06/07
Total number of decline symptomatic trees in AZ[(1)]	45.531	57.402	46.068	57.061	71.107	95.302	240.097	163.892
Critical Location of Comporta	7,42%	21,88%	49,55%	54,55%	59,82%	64,57%	60,00%	58,33%
Critical Location of Apostiça	14,34%	9,76%	3,15%	7,51%	3,12%	4,66%	5,14%	1,09%
Critical Locations (Total)	21,75%	31,64%	52,70%	62,07%	62,93%	69,23%	65,14%	59,42%
Remaining Affected Zone	78,25%	68,36%	47,30%	37,93%	37,07%	30,77%	34,86%	40,58%

[(1)] For the sake of comparison, the percentages shown refer to the Affected Zone limits stated on the Regulation n.º 1572/2003, from December, 27th, 258.000 ha.

as the percentual evolution of these numbers considering the critical locations and the reminder Affected Zone.

A general analysis of the Demarcated Area, shows that although the absolute number of symptomatic trees in the Affected Zone had increased, there has been a percentual reduction of this number in the Critical Locations, where 59, 42% of the DA decline symptomatic trees are located, as shown in Fig. 7.

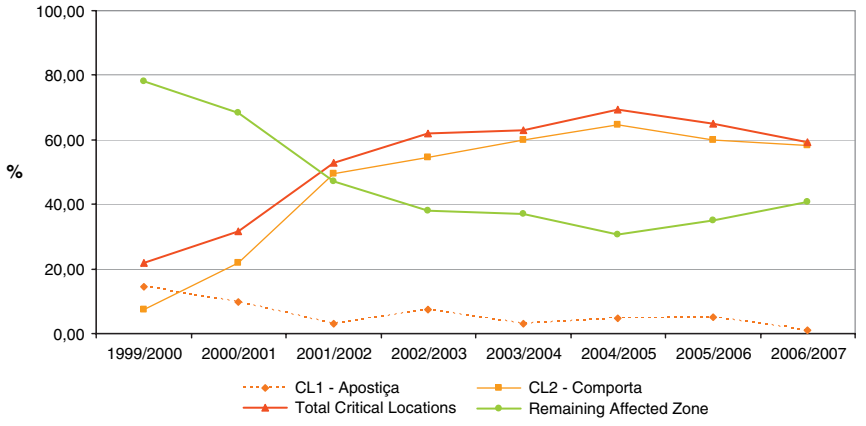

Fig. 7 Decline symptomatic trees evolution in the Demarcated Area

The 2006/2007 Survey/Eradication Campaign

A total number of 218 895 trees was identified as symptomatic, occupying 28 667 ha, from which 4 595 were located in the Clear Cut Belt. In the Demarcated Area, 214 300 decline symptomatic trees have been detected, from which 17 770 were located in the Buffer Zone and 196 530 in the Affected Zone. A total number of 5 797 samples were collected and analysed in order to screen for the presence of PWN, from which 1 232 were located in the Affected Zone, 3 703 in the Buffer Zone and 862 in the Clear Cut Belt. Details regarding the number of samples collected from symptomatic and non-symptomatic trees, at DBH height or at the canopy level and the number of samples incubated, are provided in Table 2.

In 2006/2007, 249 samples tested positive for the PWN in the Affected Zone. No positive samples were found in the Clear Cut Belt and in the Free Zone.

The total number of eradicated trees was 1 202 601, from which 218 895 were located at the Demarcated Area and 983 706 at the Clear Cut Belt; in this corridor, a large number of trees with DBH < 10 cm have been detected and eradicated (3758 054). Table 3 summarize the 2006/2007 survey and eradication campaign.

The number of eradicated trees was far beyond the number initially estimated, in what regards non-symptomatic trees. In the Clear Cut Belt there was 4 741 760 trees cut (4 041 760 trees more than initially estimated), including 3 758 054 specimens with DBH < 10.

Table 2 Number of samples collected in the different PROLUNP set regions, at Diameter at Breast Height (DBH) and at canopy level; it is also presented the number of samples incubated

N.º of samples collected	Free Zone			Demarcated Area		Clear Cut Belt
	Risk Areas (1193 plots)	Lisbon (78 plots)	200 plots	Affected Zone	Buffer Zone	
From symptomatic trees at DBH level	167	8	412	809	3 505	473
From symptomatic trees at canopy level		0	68	85	102	264
From non-symptomatic trees at DBH level	60[(1)]	37	467	297	51	113
From non-symptomatic trees at canopy level	0	0	129	41	45	12
Incubated	0	0	1.010	126	147	276
TOTAL	**227**	**45**	**1 076**	**1 232**	**3 703**	**862**

[(1)] Include 58 samples collected from material stored at Risk Areas.

Table 3 2006/2007 survey and eradication campaign results

2006/2007 campaign	Demarcated Area		Clear Cut Belt
	Affected Zone	Buffer Zone	
N.º of symptomatic trees identified	196 530	17 770	4 595
N.º of decline symptomatic trees eradicated	196 530	17 770	4 595
N.º of non-symptomatic trees eradicated	Not applicable	Not applicable	983 706 + 3 758 054

Actions Planned

Actions to be implemented will vary according with the area of intervention (Free Zone and Demarcated Area), as follows:

Free Zone Survey
Demarcated Area
Survey
Eradication
Insect Vector and Scolitids' Control
Inspection and Control
Forest Reconversion
Public Awareness
Research and Development