The Pine Wood Nematode: a personal view

by

Helen Braasch & Manuel M. Mota

The first report of the disease ("pine wilt disease") associated with the pinewood nematode, goes back to 1905, when Yano reported an unusual decline of pines from Nagasaki. For a long time thereafter, the cause of he disease was sought, but without success. Because of the large number of insect species that were usually seen around and on infected trees, it had always been assumed that the causal agent would prove to be one of these. However, in 1971, Kiyohara and Tokushike found a nematode of the genus *Bursaphelenchus* in infected trees. The nematode found was multiplied on fungal culture, inoculated into healthy trees and then re-isolated from the resulting wilted trees. The subsequent published reports were impressive: this *Bursaphelenchus* species could kill fully-grown trees within a few months in the warmer areas of Japan, and could destroy complete forests of susceptible pine species within a few years. *Pinus densiflora*, *P. thunbergii* und *P. luchuensis* were particularly affected.

In 1972, Mamiya and Kiyohara described the new species of nematode extracted from the wood of diseased pines; it was a named *Bursaphelenchus lignicolus*. Since 1975, the species has spread to the north of Japan, with the exception of the most northerly prefectures. In 1977, the loss of wood in the west of the country reached 80%. Probably as a result of unusually high summer temperatures and reduced rainfall in the years 1978 and 1979, the losses were more than 2 million m^3 per year. From the beginning, *B. lignicolus* was always considered by Japanese scientists to be an exotic pest. But where did it come from?

That this nematode could also cause damage in the USA became clear in 1979 when *B. lignicolus* was isolated in great numbers from wood of a 39 year-old pine tree (*Pinus nigra*) in Missouri which had suddenly died after the colour of its needles changed to a reddish-brown colour (Dropkin und Foudin,

1979). In 1981, *B. lignicolus* was synonymised by Nickle et al. with *B. xylophilus* which had been found for the first time in the USA as far back as 1929, and reported by Steiner and Buhrer in 1934. It had originally been named *Aphelenchoides xylophilus*, the wood-inhabiting *Aphelenchoides* but was recognised by Nickle, in 1970,to belong in the genus *Bursaphelenchus*. Its common name in the USA was the "pine wood nematode" (PWN. After its detection in Missouri, it became known that *B. xylophilus* was widespread throughout the USA and Canada. It occurred there on native species of conifers where, as a rule, it did not show the symptoms of pine wilt disease unless susceptible species were stressed eg., by high temperature. This fact was an illuminating piece of evidence that North America could be the homeland of PWN. Dwinell (1993) later reported the presence of *B. xylophilus* in Mexico.

The main vector of the PWN in Japan was shown to be the long-horned beetle *Monochamus alternatus*, belonging to the family Cerambycidae. This beetle lays its eggs in dead or dying trees where the developing larvae then feed in the cambium layer. It was already known in Japan in the 19th century but in the 1930s, it was said to be present in most areas of Japan, but was generally uncommon. However, with the spread of the pine wilt disease, and the resulting increase of weakened trees that could act as breeding sites for beetles, the populations of Monochamus spp. increased significantly In North America, other *Monochamus* species transmit PWN, and the main vector is *M. carolinensis*. In Japan, there are also other, less efficient vectors in the genus *Monochamus*. Possibly, all *Monochamus* species that breed in conifers can transmit the PWN. The occasional transmission by less efficient species of *Monochamus* or by some of the many other beetle genera in the bark or wood is of little significance. In Europe, *M. galloprovincialis* and *M. sutor* transmits the closely related species *B. mucronatus*. Some speculate that these two insect species are “standing by” and waiting for the arrival of *B. xylophilus*.

In 1982, the nematode was detected and China. It was first found in dead pines near the Zhongshan Monument of Nanjing (CHENG *et. al*. 1983); 265 trees were then killed by pine wilt disease. Despite great efforts at eradication in China, the nematode spread further and pine wilt disease has been

reported from parts of the provinces of Jiangsu, Anhui, Guangdong, Shandong, Zhejiang and Hubei (YANG, 2003). In 1986, the spread of the PWN to Taiwan was discovered and in 1989, the nematode was reported to be present in the Republic of Korea where it had first been detected in *Pinus thunbergii* and *P. densiflora*. It was though to have been introduced with packing material from Japan. PWN was advancing.

In 1984, *B. xylophilus* was found in wood chips imported into Finland from the USA and Canada, and this was the impetus to establish phytosanitary measures to prevent any possible spread into Europe. Finland prohibited the import of coniferous wood chips from these sources, and the other Nordic countries soon followed suit. EPPO (the European and Mediterranean Plant Protection Organization) made a recommendation to its member countries in 1986 to refuse wood imports from infested countries. With its Directive of 1989 (77/93 EEC), the European Community (later called the European Union or EU) recognised the potential danger of *B. xylophilus* for European forests and imposed restrictions on imports into the Europe. PWN was placed on the quarantine list of the EU and also of other European countries. Later, in 1991, a dispensation was allowed by the Commission of the EU(92/13 EEC) for coniferous wood from North America provided that certain specified requirements were fulfilled that would prevent introduction.

Helen Braasch: "The Pine Wood Nematode has been particularly attractive to me ever since I learned of the enormous damage it had caused in Japan in the 1970s. Damage by the potato cyst nematode, by *Meloidogyne* species, by the stem and bulb nematode and by other nematode pests of agricultural or horticultural importance were well known to us in Europe, but a nematode that could cause the death of great pine trees, was almost unbelievable. The only other equivalent case was that of *Rhadinaphelenchus* (*Bursaphelenchus*) *cocophilus*, the causal agent of red ring disease of palms in the Carribean Islands. At this time, I was in charge of the Quarantine Laboratory in the Central Plant Protection Office of the former East Germany (DDR) in Potsdam, close to Berlin. Being always on the lookout for new threats in plant quarantine, it was quickly clear to me that PWN in Japan represented a

major new quarantine problem. In 1983, I published, in *Nachrichtenblatt für den Pflanzenschutz in der DDR*, the first European report on PWN, entitled: "The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner und Buhrer, 1934) Nickle, 1970, from the point of view of plant quarantine (in German)".

I was concerned with PWN, in one way or another, for more than 10 years without ever having seen it. Even obtaining the relevant literature, for those of us "behind the Wall" often required it to be obtained by circuitous routes! When David McNamara, as Assistant Director of EPPO visited our institute at this time (the DDR had become a member of EPPO) I took the opportunity to ask him (quietly!) to support my participation in an EPPO Panel of Experts on PWN. What a request! When, for me, any participation in scientific meetings in the „capitalist abroad" was forbidden. My boss at the time, the head of the Central Plant Protection Office, considered my interest in *Bursaphelenchus* to be an unnecessary interference with my work as a quarantine nematologist. How often did I hear the expression: „This is not the Panel on *Bursaphelenchus*!" The belief somehow persisted that the"Iron Curtain" would keep the PWN out! But they did not reckon with my stubbornness. As I could not obtain a sample of the PWN, I concerned myself with its relatives. Thus, I discovered that *B. mucronatus*, the nearest relative of the PWN, was not only present in Germany but also frequently detected in pine timber imported from Russia; I published this in 1979.

The crucial and dangerous fact about the *Bursaphelenchus* spp. is that they can survive for a very long time within wood, together with the larval stages of their insect vectors. The sampling of the Russian sawn timber at our wood storage sites was spectacular. We found boards which looked like sieves as a consequence of the *Monochamus* infestation, and the workers at the sites spoke of "swarms of flying beetles". The beetles would be able to obtain their maturation feeding in the surrounding Brandenburg pine forests and later would be able to lay their eggs in weakened trees. The dauer larvae of *B. mucronatus,* which are mainly carried in the tracheae of the insects, would be transported to the trees where they could possibly find a new vector and perhaps mingle with native populations. This could happen in a similar way, we believed, with the PWN which would then find the required

environmental conditions to take up residence in the dry pine forests of Brandenburg where summer temperatures are relatively high for Germany.

My relationship with PWN improved with the collapse of the Berlin Wall and the unification of Germany. The first personal contact that I had thereafter with the "western world" was in the Institute for Nematology and Vertebrate Science of the BBA (Federal Biological Institution) in Münster, where Dieter Sturhan and Marlies Schauer-Blume had also been concerning themselves with the *Bursaphelenchus* problem, and had demonstrated the presence of *B. fraudulentus*. in deciduous trees. The title of their publication: „The occurrence of pine wood nematodes (*Bursaphelenchus* spp.) in the Federal Republic of Germany" (1989) led later to the misunderstanding in certain parts of the world that it was the pine wood nematode (*B. xylophilus*) itself that was present in Germany. The authors had failed to add a question mark to the title. I was later required to provide clarification to the resulting enquiries. I obtained *Bursaphelenchus* cultures from Münster and I could, at last, study the PWN "in person". New doors were opened to me and I obtained a new position in the Kleinmachnow Branch of the Federal Biological Institution for Agriculture and Forestry. During my vacation, I made the first personal contacts with Canadian 'Bursaphelenchists', being received very amicably in Vancouver by John Webster and Jack Sutherland. Now, I could also establish my membership of the EPPO Panel of Experts on the Pine Wood Nematode, which was chaired by David McNamara.

The Department for Economic and Legal Affairs in Plant Protection of the BBA in Braunschweig, with an external branch in Kleinmachnow, in which I was employed as a nematologist from 1990, had the task of adopting and fulfilling the requirements of Annexes I-V of EU Directive 77/93/EWG (Plant Quarantine Directive), which covered numerous pests, plants, plant products and other regulated articles. The requirements concerning coniferous wood from North America needed particularly intensive activity. Even if the occasional journeys to Brussels to participate in the EU

Standing Committee on Plant health were inevitably stressful due to the need to leave home at 5 o'clock in the morning and to return at about 11 o'clock in the evening, I was always interested in the statements from the representatives of other countries on the time-consuming and expensive implementation of the requirements concerning PWN.

The Sword of Damocles was hanging over Europe: coniferous wood from infested countries was still being imported, although under certain conditions. The phytosanitary measures considered necessary by the EU (especially heat treatment of wood to prevent introduction of *B. xylophilus*) were accepted only reluctantly by the exporting countries, because of the extra expenditure needed to be applied to their wood exports. Surveillance of the cargoes imported into Europe was limited to an insufficient number of samples, while the examination of documentation continued at a high rate. The danger of introduction with packing wood was not adequately recognised. Meanwhile, some experts in North Americans began to question whether the PWN could survive in Europe and, even if it did, would it cause damage anywhere there apart from the Mediterranean reagion where the temperature was sufficiently high. There were even some suggestions that the PWN might be already present in Europe and that the import restrictions were, therefore, unnecessary!

One good thing came out of this situation: although the main function of my department was specialist advice, I was also placed in the position to be able to conduct research on the PWN problem and to present the results at international conferences. This privilege was not always free from envious glances from some of my colleagues, and also some obstacles needed to be overcome, or simply ignored. I surveyed the German States for *Bursaphelenchus* species, studied their taxonomy and biology, compared the morphology and damage caused by *B. xylophilus* and *B. mucronatus*, conducted inter-species crossing experiments, researched the variability and climatic needs, the means of transmission and spread, and collaborated with foreign nematologists. With John Philis from Nicosia, I studied the *Bursaphelenchus* fauna of Cyprus; *B. xylophilus* was, luckily, not found there, despite patches of dead pines. From a damaged pine in South Africa, an isolate of *Bursaphelenchus* was sent to me by A. Swart which, again fortunately, proved not to be *B. xylophilus*, but *B. leoni*.

Several international conferences between 1994 and 2004, with sections on pine wilt disease, all contributed not only to the international exchange of experience with the *Bursaphelenchus* problem, but also led to the foundation of a pan-European research collaboration on *Bursaphelenchus*. This collaboration reached its high-point at the end of the 20th century with the completion of the EU Project RISKBURS (1996-2000). During a visit to our partner in the research project in Vienna, I met Heinrich Schmutzenhofer, Secretary of the International Union of Forestry Research Organisations (IUFRO). In 1981, Dr. Schmutzenhofer had reported finding a *Bursaphelenchus* species associated with decline of fir trees in Austria; he made available to me the nematode material that he had collected at the time. During the 'Symposium on sustainability of pine forests in relation to pine wilt and decline' in Tokyo, Japan (1998) I was able, for the first time, to hold discussions with Yasuharu Mamiya – an impressive, modest and yet radiating personality.

In the 1990s, it was recognised that a scientific response should be given to the question of how dangerous would the PWN really be to Europe and to try to convince the doubters, especially in North America, of the need for the EU's quarantine measures. The EU, therefore, established an Expert Group from the member states under the leadership of Hugh Evans of the U.K. The results of the Group's deliberations were published in 1996 in the EPPO Bulletin as a formal pest risk analysis (Evans, H. F.; McNamara, D. G.; Braasch, H.; Chadoeuf, J.; Magnusson, C.: Pest Risk Analysis (PRA) for the territories of the European Union (as PRA area) on *B. xylophilus* and its vectors in the genus *Monochamus*). The most important conclusions of this analysis were that the whole of the PRA area is suitable for colonisation by *B. xylophilus,* but that the dry Mediterranean and continental regions would be in particular danger for the occurrence of pine wilt disease, that the occurrence in Europe would have important economic consequences, and that the greatest danger from wood imports would be when the nematode and its vector were both present zt the same time. The final conclusion was that shipments of coniferous wood from infested areas required phytosanitary measures.

The EU-financed project RISKBURS, of which I was the project leader, included biologists, forest scientists and molecular biologists from Germany, Greece, Ireland, Italy and Austria. The project allowed, for the first time in Europe, large-scale detection surveys to be conducted to determine the *Bursaphelenchus* species present in Europe. Fortunately, *B. xylophilus* was not detected in the surveyed areas in Germany, Greece, Italy and Austria. Numerous pathogenicity tests conducted on young conifer plants with 15 *Bursaphelenchus* species suggested that *B. mucronatus* and *B. sexdentati* could also have a potential for pathogenicity, although, so far, no confirmation of these laboratory data have been observed on forest trees. The collaboration between Wolfgang Burgermeister and his colleagues with myself , which had begun before the start of the project, on the molecular characterization of *Bursaphelenchus* spp., proved to be fruitful. The characterization of species that had previously been identified morphologically, first used RAPD-PCR, later ITS-RFLP and finally sequencing. The methodology provided an essential contribution to the results of the project and then, later, to the confirmation of the first introduction of *B. xylophilus* into Portugal; the methods are still used today.. Techniques for molecular identification of *B. xylophilus* und *B. mucronatus* were also elaborated in Ireland, France und North America. The reference pictures built up in Germany, with the aid of the extensive BBA collection of *Bursaphelenchus* cultures, permitted the differentiation of *B. xylophilus* from about 30 other *Bursaphelenchus* spp. by means of the ITS-RFLP technique."

Manuel Mota: "In 1999, *B. xylophilus* was detected for the first time within the territory of the EU, more precisely in an area in the Setúbal Peninsula in Portugal. I am proud to have led a team of researchers from Portugal, which included Maria Antónia Bravo and Edmundo Sousa, from INIA. The discovery was made in May 1999 while we were surveying cerambycid beetles and associated aphelenchid nematodes, in the area of Pegões, a town located in the Setúbal Peninsula, 30 km southeast of Lisbon. In collecting samples of wood and insects, our intention was to establish which species of nematodes were present and with which species of trees and insects they were associated. We had no thought that *B. xylophilus* might be present. To our great surprise (and alarm), one of the samples yielded a tremendous number of specimens of a species of *Bursaphelenchus*. It was my M.Sc.

student, Ana Catarina Penas who made this observation during research work for her thesis, and she called me to confirm. We also asked Maria Antónia Bravo if she had seen the same nematode in her lot of samples and she said 'yes'. The nematode appeared to us to be *B. xylophilus* but we needed a confirmation as soon as possible. So, so we contacted Helen Braasch and Wolfgang Burgermeister in Germany who, more than anyone else, had the expertise to confirm quickly this initial diagnosis, by molecular biology (ITS-RFLP).

Helen Braasch: "The greatest surprise of my nematological life was when I looked through my microscope in Spring 1999 at a sample from a killed pine tree (*Pinus pinaster*) which had been sent to me by Manuel Mota from the University of Évora, Portugal, and saw thousands of wriggling specimens of *B. xylophilus*. The Pine Wood Nematode in Europe! It was hard to believe. Morphologically, there was little doubt, but a molecular study would confirm the identification. Within a week, we were able to confirm Manuel's suspicions

Manuel Mota: "The results arrived back from Germany with the message that the molecular analysis confirmed our worst fears: we had detected pine wood nematode for the first time in the EU! Following a team meeting, we proceeded to inform our institutional authorities (University president, Research directors) about this issue and immediately contacted the national plant protection authority (DGPC), who would have to inform the EU Commission in Brussels about this. The initial intention of the EU, in September 1999, was to impose a general embargo on pine wood exports from Portugal to other countries of the EU. Fortunately, and following some intense political lobbying, the quarantined area was restricted to the Setúbal Peninsula. On a short anecdotal note, I remember my friend José Francisco Fernandes, owner of a large property inside the affected area, who in the past had joked and pulled my leg for studying these strange little animals under the microscope, with no apparent practical interest. Well, once his pine stand was subject to strict and expensive quarantine measures, he suddenly became very interested in nematology, and wanted to learn everything about this new "enemy" which caused major economic damage to his financial situation!

Helen Braasch: "I felt that I must go to Portugal as soon as possible, and, shortly thereafter, I travelled there with Wolfgang Burgermeister and Kai Metge. We were met at the airport by Manuel Mota and a delegation of important people and very soon, during a working lunch, we exchanged our information. I insisted on seeing the infected tree and, after the meal we drove on the Setúbal peninsula towards Évora to the affected forest plot. Of course, it was not really an amusing situation but we laughed when Manuel could not, at first, find the infected tree! After walking back and forth along the forest path, the tree was at last found : It had been sawn down and the stump was still standing; the tracks of beetle larvae could be seen on it. We were shown a photograph of the tree as it appeared while still standing, but it looked very different now.. As we stood there in sad discussion around the remains of the fallen tree, I heard the repeated expression from the representative of the Portuguese Ministry: "It's a nightmare"! Samples taken from the site, from the stump and from nearby trees confirmed the infestation. As well, in a second area lying close to a timber storage site, we made another find.. A forest worker had noticed that the pines appeared to die unusually quickly.

The results of the sampling did not permit any doubt, and they soon appeared in print: Mota, M. M.; Braasch, H.; Bravo, M. A.; Penas, A. C.; Burgermeister, W.; Metge, K.; Sousa, E. (1999). First record of *Bursaphelenchus xylophilus* in Portugal and in Europe.Nematology **1** (1999), 727-734. Shortly afterwards, the vector was recognised to be *M. galloprovincialis* which had previously been found only rarely in Portugal. The discovery of PWN in Portugal dramatically altered the European view of the problem. The EU quarantine machinery rolled into action and the Portuguese did their best to destroy any infected trees, delimit the infested area, and prevent the spread of the pest to other areas of Portugal and Europe. Although spread of the pine wilt disease has been prevented, it has proved impossible to eradicate the PWN infestation, despite the felling and destruction of all suspected trees in the demarcated zone and the buffer zones.

The EU carried out inspection visits to evaluate the situation in Portugal. During the first inspection visit, we were pursued by the paparazzi, but our delegation was not prepared to speak to the press. The press photographers succeeded in taking pictures and, on September 16th, an article appeared in the

Portuguese press entitled: '*Inspectores Europeus em Silencio'* ('The European Inspectors remain silent'). It included a picture of our delegation, under which my name was given with the additional information that it was I who had confirmed the presence of the pest in Portugal.
I had hardly returned to Kleinmachnow before the telephone began to ring incessantly: the European press wanted to know something about the PWN. The German Press Agency spread the news about the occurrence of *B. xylophilus* in Europe. The press reports ranged from more or less scientifically correct to rather distorted, and had headlines such as: "New Pest spreads Shock and Awe", "The Advancing Worm", "The Invasion of the Worms", "Pine Stands in Portugal Dying of Thirst", "Worm becomes a Global Threat", "Tiny Worm Destroys Complete Pine Forests", "Scientist from Kleinmachnow Protects the World's Coniferous Forests"– and these are just a few of the many headlines.

According to EU Directive 2000/29/EC, which replaced the earlier Directive 77/93 EEC, the most important quarantine requirements for imported conifer wood from infested countries were (depending on the commodity type) heat treatment (56°C in the centre of the wood for at least 30 minutes) , debarking, drying and freedom from bore holes (exit holes of beetles). Only the last three requirements were applied to coniferous packing wood, which is frequently used to contain and support other commodities and which is often of inferior quality; this proved to be a serious mistake. There is considerable circumstantial evidence that PWN was probably introduced to Portugal with packing wood from China. The ports of Setúbal and Lisbon are both near to the infested zone and there is a strong suspicion that infected wood passed through these ports. The impression is certainly strengthened that importation of packing wood played a key role in the spread of PWN. For example, in China, saw mills, building sites and storage areas for packing wood are considered to be sources for new infestations. In recent years, in many European and Asiatic countries, samples were taken from packing wood coming from infested areas, and PWN and living larvae of the vector beetles were found. This is the greatest danger; both organisms together in the wood!

In Brussels, the experts from the member states discussed how the sorry situation in Portugal should be combated. These meetings are among the most interesting memories of my professional career. It required intense concentration to ensure that the right thing was done, nothing should be overdone, nothing should be neglected, and that the correct procedures should be completed. The decision of the EU of January 11th, 2000 (2000/58/EG) and its modified version of March 12th, 2001 (2001/218/EG), as well as later adjustments, established the regulations whereby the transfer of the pest with coniferous wood and plants from the infested zone to pest-free areas in Portugal and in other countries should be prevented. At the same time, the member states of the EU were obliged to conduct surveys in their countries to officially determine the actual distribution of PWN. The diagnostic protocol for *B. xylophilus*, which had been developed by EPPO, would facilitate identification. Many of the non-EU countries in Europe also decided to examine their pine stands. The results of our research from the previous years indicated that *B. xylophilus* had not, at that time, been found in any European country."

In order to prevent further introductions of the dangerous pest into Europe with packing wood, the EU Commission decided, on March 12th,, 2001, to apply immediate but temporary measures (2001/219/EG) requiring packing wood from Canada, USA, China and Japan to be specifically treated by heat, fumigation or pressure impregnation and to be marked by the exporting country to show the origin and treatment applied. Consignments from China had to be accompanied by a phytosanitary certificate indicating the origin of the packing wood and the identity of perpetrator of the measures carried out. Furthermore, the member state receiving the consignment must confirm by sampling that the required conditions have been met.

But these requirements would not be sufficient. It has long been clear that packing wood is often re-used and, therefore, moves around the world. And that *Bursaphelenchus* spp. survive such travel, as do many other pests. Because the origin of wood packaging material is often difficult to determine, globally applied measures are necessary in order to significantly reduce the risk of pest spread. FAO Guidelines for Regulating Wood Packaging Material in International Trade (ISPM 15: FAO, 2003) are recognized as the basis for phytosanitary measures applied by members of the World Trade

Organization (WTO); non-contracting parties are also encouraged to observe these standards. These standards describe phytosanitary measures to reduce the risk of introduction and/or spread of quarantine pests associated with packaging material (including dunnage, crating, pallets, packing blocks, drums, cases *etc*.) made of coniferous and non-coniferous raw wood, in use in international trade.

Helen Braasch: "After my retirement, I conducted a course in China on the identification of *Bursaphelenchus* spp., together with Wolfgang Burgermeister and Thomas Schröder of the BBA Braunschweig; this took place in October 2002 in Shanghai and Nanjing, and was organised by Professor Maosong Lin of Nanjing. The course led to the development of useful collaboration with several Chinese scientists, especially with Jianfeng Gu of the Technical Centre, Ningbo Entry-Exit Inspection and Quarantine Bureau, Ningbo, Zhejiang, China. Almost all wooden packages imported through Ningbo harbour have been sampled and inspected since 1997. *Bursaphelenchus. xylophilus* has been detected many times in large numbers in wood samples from different countries, and a considerable number of other *Bursaphelenchus* species, among them several undescribed species, were also found. The results are alarming: The percentage of batches of packing wood containing any nematodes averaged 21.3% (between 2000 and 2005), despite the claims on the accompanying phytosanitary certificates that the wood had been heat treated. The fact of recording *B. xylophilus* in 40 (1.2%) out of 3416 samples from eleven different countries or regions, including six countries where the pinewood nematode is not known to occur, underlines the necessity of rigorous application of international agreements on the phytosanitary treatment of packing wood in international trade. Furthermore, the effectiveness of the quarantine measures agreed upon should be investigated and their application should be controlled more intensively.

In recent years several new *Bursaphelenchus* species from East Asia have been added to the already large number within this genus. Is it possible that we may find more species of *Bursaphelenchus* that are pathogenic to trees? An exampleof the difficulty of disease detection and diagnosis occurred in

Vietnam (Lam Dong province) where symptoms similar to pine wilt dissease, as well as dead *Pinus kesiya* trees were found in several locations, and were apparently associated with a *Bursaphelenchus* species (EPPO Reporting Service 2003). The disease was found in four locations in Lam Dong province in 36-48% of the trees. The species found in Vietnam is morphologically distinct from *B. xylophilus* but shares the same vector, *M. alternatus*.

Mauel Mota: "In 2001, my colleagues and I organized a scientific meeting in Évora, Portugal, for the international community working on the pine wood nematode in order to exchange views on recent research and to discuss control measures. About 50 researchers from 14 countries attended the symposium. It was noted that almost all pine species native to North America are highly resistant to pine wilt disease, whereas two of the most common pine species native to Europe, for instance *Pinus sylvestris* and *Pinus pinaster*, are highly susceptible to the disease and occur in the hotter, southern region. The pine wilt disease is a threat to pine forests in southern Europe, and the predicted climatic changes, such as increasingly warm and unusual weather conditions, may significantly influence the incidence of pine wilt disease. The conclusions arrived at in Évora indicated that the final resolution for controlling pine wilt should rely on eradication of the nematode or resistance-breeding strategies. Control measures should be aimed at breaking the pine tree/pine wood nematode/pine sawyer disease triangle. Spraying of insecticides, trunk injection, cutting and destroying of trees presumed to be infected, restrictions in transporting wood, heat treatment and fumigation of lumber are the control measures most applied in countries with pine wilt disease. In spite of these various efforts, however, the total amount of pine timber lost to the disease is not decreasing conspicuously. This is also true for the newly invaded region in Portugal. Therefore, strong quarantine measures and a particularly rigorous response at the early stage of any new occurrence of the pine wood nematode are essential to hinder the spread of this economically important disease in Europe.

A better understanding of the inter-relationships between the nematode, its vectors and the host trees is clearly a precondition for limitation of damage by *B. xylophilus*. Additionally, an improvement in pest risk analysis systems would help to prevent further spread and, hopefully, the continuing EU project 'Development of improved Pest Risk Analysis techniques for quarantine pests, using pine wood

nematode, *Bursaphelenchus xylophilus*, in Portugal as a model system' (PHRAME) will refine PRA techniques. Future symposia of the leading scientists working in this field will give us ideas for better managing the problem in all its aspects.

Helen Braasch: "The pine wood nematode has not lost its fascination for me and I hope to be able to add to the 70 scientific papers that I have published on the genus *Bursaphelenchus*. Teamwork is essential in today's world and I would like to take this opportunity to recognise especially the outstanding collaboration with colleagues from the BBA in Braunschweig (Wolfgang Burgermeister, Thomas Schröder, Kai Metge), the Plant Protection Service of Brandenburg (Ute Schönfeld), The Federal Forestry in Vienna, Austria (Martin Brandstetter), the Chinese Quarantine Service in Ningbo (Jianfeng Gu) and with David McNamara. As well, the creation of the extensive data base by Manuel Mota and his colleagues is of great worth.