STATUS OF A PINE WILT NEMATODE IN VIETNAM

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ABSTRACT

Pinus kesiya Royle ex Gordon plantations are of major economic, social, and environmental importance to the Lam Dong province of Vietnam. Pine wilt symptoms and dead trees are found in pine plantations at various locations. A systematic survey was needed in order to identify the nematode and its vector, the Japanese sawyer beetle *Monochamus alternatus* Hope, and to evaluate the incidence and severity of the disease. The health status of the trees (disease incidence) was evaluated by measuring the amount of oleoresin exudation after holes were punched in pine stems. Experimental plots within pine stands were typically 400 m^2. Disease incidence was assessed as 36.5%, 46.4%, 47.9%, and 48.0% in Lang Bian, Phi Lieng, Cam Ly, and K'Long K'Lanh respectively. The death rate of pine trees increased annually and was dependent on the density of plantations. Yearly losses in the high density plantations were higher than in the lower density plantations. Nematodes extracted from wood samples were identified as a species of *Bursaphelenchus*. The recovery of nematodes from affected trees varied between specimens collected from different locations. The highest densities of nematodes were found in samples collected at K'Long K'Lanh and Cam Ly. Based on the morphological characteristics of adult sawyer beetles, all were identified as the Japanese sawyer beetle. Sawyer beetles have two emergence periods each year, the first lasting from the end of March to early July and the second from September to the end of October. The largest numbers of adults emerged at the end of April and at the end of September. Stems of 3-year-old *Pinus kesiya* were inoculated with cultured nematodes and the pathogenicity of the *Bursaphelenchus* sp. was demonstrated.

Keywords: *Bursaphelenchus* sp.; *Monochamus alternatus*; *Pinus kesiya.*

INTRODUCTION

Plantations and natural forests of *Pinus kesiya* are distributed mainly in Lam Dong province, in the south-central highlands of Vietnam. The elevation of these forests is from 900 to 1500 m above sea-level. Annual average maximum temperature is 23.3°C, annual average minimum temperature is 14.3°C, and annual average rainfall is about 1730 mm.

Pine wilt disease was discovered for the first time in Lam Dong during 1994, and since that time the incidence and severity of the disease have increased. Reports from provincial forest officers indicate that about 1000 ha of pine plantations have tree wilt symptoms.

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Disease incidence approaches 40–50% at some locations — for example, Cam Ly and K'Long K'Lanh.

The disease has attracted considerable attention from scientists and forest management organisations. Since August 1999, surveys have been implemented, based on standardised plots laid out at different locations in Lam Dong province, in order to determine the cause of tree death. Wood samples have been collected from dead trees for nematode extraction and log samples have been collected from infected plantations and natural forests for life-cycle studies of pine sawyer beetles and for the identification of nematodes and their vectors. The health status of trees was evaluated by measuring oleoresin exudation resulting from standardised wounds inflicted on pine stems.

The results of this survey have great significance in providing the basic information and scientific background for minimising disease impacts on plantations of *P. kesiya* in Vietnam.

MATERIALS AND METHODS

Surveys of disease incidence were conducted in Lam Dong province in August 1999 and January 2000.

Plots measuring 400 m^2 were laid out at Lang Bian mountain, Lac Duong district; Phi Lieng, Lam Ha district; K'long K'lanh, Lac Duong district; and Cam Ly, Da Lat city. The health status of trees was evaluated by assessing the amount of oleoresin exudation from a hole 12 mm in diameter punched in pine stems. The following categories were applied:

- A: Abundant resin exudation
- B: Little resin exudation
- C: Fresh inner bark, but no oleoresin
- D: Dead trees.

Reduction and cessation of oleoresin exudation is the first detectable symptom of pine wilt. Japanese entomologists have discovered a very close relationship between reduction and cessation of oleoresin exudation from pines tree in early summer, and the death of such trees in the following autumn (Mamiya 1983).

Wood chip samples from dead trees for nematode extraction, and wood log samples for rearing sawyer beetles, were collected within the experimental plots and from the other locations mentioned above.

Wood chips were submerged in sterile water held in funnels. After 24 hours, about 15–20 ml of the water were collected and nematodes were observed through a stereo-microscope. The number of nematodes present per 5 g wood chips was compared among specimens collected from various locations. Collected nematodes were kept in 1% formaldehyde solution for taxonomical comparison and further studies.

Log samples from affected stands were stored in insect-proof enclosures at the Forest Science Institute of Vietnam (FSIV) laboratories in Hanoi to allow insect emergence. The logs were observed each day and dates of emergence of pine sawyer beetles were recorded. Emerged adult sawyer beetles were collected and identified.

Due to the limited dispersal of fourth instar larvae, cultured nematodes were used in inoculation experiments. To obtain large quantities of nematode for artificial inoculation

experiment, it is best to culture *Bursaphelenchus* sp. Twenty nematodes, including male and female, were put on a *Botrytis cinerea* mat on PDA medium in a 500-ml flask. Each flask could hold about 25 000 nematodes after 2 weeks.

Artificial inoculation of 3-year-old *Pinus kesiya* was attempted using six treatments with different nematode concentrations per tree — namely, 1000, 2000, 3000, 4000, 5000 nematodes per tree, and a control treatment (water only); 10 trees for each treatment.

RESULTS AND DISCUSSION

Disease Incidence

Evaluation of the health status of trees (disease incidence) was conducted on experimental plots at different places by measuring the oleoresin exudation from wounded pine stems using the four criteria described above.

The results (Table 1) showed that the health status of trees in pine plantations varied between different sites. Trees with symptoms from categories B to D were considered to be nematode-infected. Disease incidences were 36.5 %, 46.4%, 47.9%, and 48.0% in Lang Bian, Tuyen Lam, Cam Ly, and K'long K'lanh respectively.

TABLE 1–The health status of trees (disease categories) at four experimental sites in Lam Dong Province

Health status*	Plot 1 Lang Biang		Plot 2 Tuyen Lam		Plot 3 Cam Ly		Plot 4 K'long K'lanh	
	Disease category	%	Disease category	%	Disease category	%	Disease category	%
A	40	63.5	30	53.6	37	52.1	39	52.0
B	11	17.5	11	19.6	12	16.9	9	12.0
C	3	4.7	4	7.2	8	11.3	10	13.3
D	9	14.3	11	19.6	14	19.7	17	22.7
Total	63	100.0	56	100.0	71	100.0	75	100.0

* A = abundant resin exudation
B = little resin exudation
C = fresh inner bark, but no oleoresin
D = dead trees

Yearly Losses of Pine Trees Infected by Wood Nematodes

Two permanent plots (dimension 10 000 $m^{2)}$ were laid out in Cam Ly in 10-year-old plantations . Stand stocking in plot No. 1 was 1650 stems/ha and in plot No. 2 it was 1250 stems/ha. Yearly losses of pine trees infected by wood nematodes are presented in Table 2.

The rate of tree deaths increased annually in both plots from 1998 to 2000. Rate of tree death appeared to be partially dependent on stand density, with more tree losses in the more heavily stocked stand; however, a more comprehensive study would be needed to test this hypothesis.

TABLE 2–Yearly losses of pine trees infected by wood nematodes in two experimental plots at Cam Ly

Year	Plot 1		Plot 2	
	Tree deaths	%	Tree deaths	%
1994–97	107	6.5	97	7.8
1998	38	2.3	18	1.4
1999	59	3.6	35	2.8
2000	67	4.1	43	3.4
Total	271	16.4	193	15.4

Nematode Extraction

In January 2000, a further survey in Da Lat city was carried out. Pine wood nematodes were found in all collected wood chip samples. Detailed results are shown in Table 3.

The density of nematodes varied between specimens collected from various locations. The highest numbers of nematodes were found in specimens collected at Cam Ly and K'Long K'Lanh, and the largest number of nematodes in a single specimen was found in a tree that had just died.

TABLE 3–Numbers of nematodes in wood chip samples collected in Lam Dong province

No.	Specimen No.	Tree dbh (cm)	Tree height (m)	Tree age (years)	Health status	Location	Numbers of nematodes extracted
1	6	20	7.0	16	Dead 1999	Cam Ly	682
2	7	5	3.0	4	Dead 2000	Cam Ly	2 828
3	8	6	3.0	3	Dead 2000	Cam Ly	242
6	11	13	13	15	Dead 1999	Cam Ly	58
8	13	15	9.0	15	Dead 2000	Cam Ly	2 871
9	14	6	2.5	4	Dead 2000	Cam Ly	44
10	15	10	3.0	8	Dead 2000	Cam Ly	754
11	16	18	5.0	7	Dead 1999	Cam Ly	58
12	17	15	5.0	7	Dead 1999	Cam Ly	20
13	18	10	5.0	7	Necrosis of needles 40%	Cam Ly	40
14	19a	10	3	7	Necrosis of needles 30%	Cam Ly	21 301
17	21	13	6.0	7	Dead 1999	Cam Ly	290
18	22	6	5.0	7	Dead 1998	Langbian	1 305
22	26	10	6.0	5	Necrosis of needles 80%	Langbian	841
26	30	8	4.0	6	Dead 1998	Langbian	363
27	31	7	5.0	5	Dead 1999	Lam Ha	470
28	32	7	5.0	5	Dead 1999	Lam Ha	1 390
29	33	7	3.5	5	Dead 1999	Lam Ha	20
30	34	6	4.0	5	Dead 1999	Lam Ha	10
31	35	10	6.0	5	Necrosis of needles 50%	Lam Ha	20
32	36	10	6.5	5	Dead 2000	Lam Ha	550
34	38	10	6.5	7	Dead 2000	Klonglanh	300
35	39	7	6.0	7	Dead 2000	Klonglanh	540
38	42	10	7.0	7	Necrosis of needles 98%	Klonglanh	8 580

Nematode Taxonomy Study

Specimens were collected for nematode extraction at Cam Ly, Lang Bian, Phi Lieng, K'long K'lanh, and in a natural forest in Lam Dong province. The morphological and anatomical characteristics of males and females are described in Table 4.

TABLE 4–Average measurements and ratios, with range

Length and ratio	Male		Female	
	Nematode from Lam Dong	*B. xylophilus**	Nematode from Lam Dong	*B. xylophilus**
Body length (μm)	562.1 (356.3–811.8)	730 (590–820)	572.7 (360.8–820.8)	810 (710–1010)
Spicule length (μm)	14.2 (11.3–15.8)	27 (25–30)		
Head to vulva × 100/body length			71.8 (68.3–75.3)	72.7 (67.0–78.0)
Length/width	28.8 (23.2–38.1)	42.3 (36–47)	29.4 (23.6–37.3)	42.3 (36.0–47.0)
Body length/length from head to oesophagus	11.8 (8.9–18.5)	9.4 (7.6–11.3)	12.1 (9.0–15.7)	9.4 (7.6–11.3)
Body length/tail length	20.9 (16–27.4)	26.4 (21–31)	12.5 (8.9–15.4)	26.0 (23.0–32.0)
Tail length/width at anus	2.2 (1.7–2.8)		4.2 (3–5.40	
Stylet length (μm)	12.8 (9.5–14.4)	14.9 (14.0–17.0)	12.3 (9.0–15.3)	15.9 (14.0–18.0)
Uterus length (μm)			66.5 (45.0–97.2)	

* Data from Mamiya & Kiyohara (1972)

Female: body is curved towards the ventral surface after killing by heat.Vagina is cylindrical without a vulval flap; uterus is long and usually 3–4 times body width at the vulval position. The length from vulva to anus is 7–8 times longer than the body width, at the vulval position. Tail is pyramidal in shape with a small bursa at the end, and curved towards the ventral surface.

Male: body is J-shaped after killing by heat and the tail is curved towards the ventral surface, with a small bursa at the end. Spicule shape is curved. Rostrum is pointed and without cucullus. Condylus is hemispherical.

Based on the morphological and anatomical characters listed above, the nematode extracted from infected trees in Lam Dong province belongs to the genus *Bursaphelenchus*. The measurements detailed in Table 4 and the spicule shape, however, indicate that the wood nematode extracted from infected trees in Lam Dong province does not belong to any previously described species of *Bursaphelenchus* (Tarjan & Baeza Aragon 1982)

Study on Vector of the Nematode

Taxonomy study

Log samples containing larvae were collected in August 1999 and in January 2000 at the various sites and taken to FSIV for rearing. Based on the morphological characteristics of sawyer beetles emerging from pine log samples, all specimens identified by Dr Yamane of Nihon University, Japan, were Japanese sawyer beetles, *Monochamus alternatus* (Cerambycidae, Coleoptera).

*Emergence of **Monochamus alternatus***

The time-table of emerging adults of sawyer beetles is shown in Table 5. The results reported and additional data collected from the field showed that there are two generations per year. The time for life-cycle completion of the first generation is about 210–230 days, and that of the second generation is about 130–140 days. The first emergence period lasted from the end of March to early July, with peak emergence at the end of April. The second occurred from the September to the end of October, with the largest numbers of sawyer beetles emerging at the end of September. In natural conditions, the time of adult occurrence varies from year to year and from area to area (Yamane 1981). In trials the time of emergence, however, is strongly influenced by the conditions within the cages where the logs are kept (Kobayashi *et al.* 1984). Previous results of Japanese scientists showed that adult emergence started from early to late May in the Kyushu district, from mid May to early June in the Kansai district, from late May to mid June in the Kanto district, and from late June to early July in the Tohoku district. The time span of emergence was usually within 2 months (Kishi Yoichi 1995).

TABLE 5–Results of rearing sawyer beetles from pine log specimens

Specimen No.	Location	Date of sample collection	Date of emergence of sawyer beetles	Numbers of sawyer beetles
1-00	K'Long K'Lanh	24 January 2000	25 March 2000	1
2-00	K'Long K'Lanh	24 January 2000	26 March 2000	1
3-00	K'Long K'Lanh	24 January 2000	1 April 2000	1
4-99	Lang Bian	8 August 1999	20 September 1999	4
5-99	Lang Bian	8 August 1999	21 September 1999	4
6-00	Lang Bian	25 January 2000	26 March 2000	1
7-00	Lang Bian	25 January 2000	2 April 2000	1
8-00	Lang Bian	25 January 2000	5 April 2000	1
9-99	Cam Ly	7 August 1999	22 September 1999	2
10-99	Cam Ly	7 August 1999	23 September 1999	1
11-00	Cam Ly	26 January 2000	27 March 2000	1
12-00	Cam Ly	26 January 2000	13 April 2000	1
13-00	Cam Ly	26 January 2000	15 April 2000	1
14-99	Tuyen Lam	9 August 1999	25 September 1999	2
15-99	Tuyen Lam	9 August 1999	1 October 1999	3
16-00	Cam Ly	18 April 2000	15 June 2000	4
17- 00	Cam Ly	18 April 2000	27 June 2000	1
18-00	Cam Ly	18 April 2000	2 July 2000	1

Artificial Inoculation

The trees were inoculated on 2 October 2000 and after 4 months nine of the trees in Treatment 5 wilted and died, all having received 5000 nematodes. None of the treees in the other five treatments died.

REMARKS AND CONCLUSIONS

- The pine wilt nematode disease in Lam Dong province is increasing both in disease incidence in affected areas and in extension to new areas.Tree health status, as evaluated by measuring the amount of oleoresin exudation, varied in different locations with disease incidences of 36.5%, 46.4%, 47.9%, and 48.0% in Lang Bian, Phi Lieng, Cam Ly, and K'Long K'Lanh respectively.
- The cause of the pine wilt disease in Japan, China, and North America was determined several decades ago as being *Bursaphelenchus xylophilus* (Steiner & Buhrer) (Mamiya 1988). The nematode extracted from wood samples collected in Lam Dong province,Vietnam, and which was very commonly found in affected pine plantations, is clearly a species of *Bursaphelenchus*. However, it was neither *B. mucronatus* Mamiya & Enda nor *B. xylophilus*, nematodes formerly associated with pine wilt.
- The pine sawyer beetle *Monochamus alternatus* was found in both infected trees and dead trees. Adults emerging from logs placed in screen cages were intensively observed in the FSIV laboratory in Hanoi. Sawyer beetles had two generations per year with two distinct emergence periods. The first lasted from the end of March to early July, and the second from September to the end of October. The results of this survey have great significance in providing basic information and scientific background to minimise disease impacts in plantations of *Pinus kesiya* in Vietnam.
- Preliminary results of artificial inoculation of trees with cultured nematodes have shown that this newly discovered but so far undescribed *Bursaphelenchus* sp. is pathogenic to *P. kesiya.*

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