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Occurrence and distribution of the nematode *Bursaphelenchus mucronatus* in the Russian Far East

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Summary. During the summer of 1991-1993 the conifer forests of the Russian Far East area were surveyed to determine the presence of the pinewood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle 1970. Wood samples from branches, logs, and tops of dead and dying pine (*Pinus sylvestris* L., *P. koraiensis* Sieb. & Zucc.), spruce (*Picea koraiensis* Nakai, *P.ajanensis* Fisch.), larch (*Larix dahurica* Turcz.) and fir (*Abies nephrolepis* Maxim.) were assayed. The results showed that *B. xylophilus* was not present in the area, but a closely related nematode *B. mucronatus* was widespread. Morphological features of *B. mucronatus* from Primorsky territory are illustrated, the nematode distribution is given. *B. mucronatus* was obtained only from wood of plantation-grown Scots (*P. sylvestris*) and Korean (*P. koraiensis*) pines. The maximum number of *B. mucronatus* was found 20,000 nematodes per gram of pine (*P. koraiensis*) fresh wood. Most of infected *B. mucronatus* pines died during one summer, suggesting that *B. mucronatus* caused their death.
Key words: Russia, Far East, pinewood nematode, *Bursaphelenchus xylophilus, B. mucronatus*, conifers, *Pinus sylvestris, P. koraiensis*.

Pine wilt disease, caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle 1970, is a major disease of Japanese black pine (*Pinus thunbergii* Parl.) and Japanese red pine (*P. densiflora* Sieb. & Zucc.) in Japan and small localized areas in Eastern China, particularly near Nanjing (Mamiya, 1984; Yang & Wang, 1989). The nematode is also widely distributed in North America where native conifers are more or less resistant. However, pine wilt kills some exotic trees, such as *Pinus sylvestris* L., and *P. nigra* Arn. in the U.S.A. (Rutherford & Webster, 1987; Rutherford et al., 1990). Transmission of the PWN from tree to tree occurs either during maturation feeding of adult cerambicid beetles, primarily of the genus *Monochamus*, or during their oviposition (Linit, 1988; Wingfield, 1983).

In 1984 the Finnish Plant Quarantine Service found the PWN in conifer wood chips being imported into Finland from North America (Rautapaa, 1986). Because of the potential threat of pine wilt to their conifer forests, many European and some Asian countries placed an embargo on all raw softwood shipments from those areas of North America and elsewhere where the PWN occurs (Bergdahl, 1988).

Since the PWN is also a potential threat to conifer forests in much of Russia (Kulinich & Kolossova, 1993), a similar quarantine was imposed by Russian authorities in 1991. Based on climate and the fact that much of the forest area of the Russian Far East is comprised of conifers, this area is under the greatest threat from pine wilt diseases.

Consequently, in 1991 a research programme was begun at the Institute of Parasitology of Russian Academy of Sciences on the taxonomy, distribution, pathogenicity and hosts of the PWN, and closely related nematode species in Russia (Kulinich, 1993).

This paper gives the results of a survey made by the Institute of Parasitology, Moscow, and the Soil and Biological Institute, Vladivostok, Russian Academy of

Sciences for the PWN and other closely related species of *Bursaphelenchus* in the forests of the Russian Far East.

MATERIALS AND METHODS

Branches, logs, and tops from dying and dead-standing trees were collected from several localities in the Primorsky territory of the Russian Far East. Sampling was done from July to September over a 3 year (1991-1993) period. A total of 230 wood samples from pine (*Pinus sylvestris* L., *P. koraiensis* Sieb. & Zucc.), spruce (*Picea koraiensis* Nakai., *P. ajanensis* Fisch.), larch (*Larix dahurica* Turcz.) and fir (*Abies nephrolepis* Maxim.) were collected. Some wood samples were analyzed immediately in the field, while others were brought to the laboratory where they were cut into small-diameter pieces and a 5 g (wet weight) subsample from each was placed in a Baermann funnel at 20 - 24° C for 24 h for extraction of the nematodes.

To obtain populations for identification nematodes were either placed on to freshly cut, 5 - 7 cm diameter, disks of healthy Scots pine (*Pinus sylvestris* L.) wood and kept at 27° C, or cultured on the fungi *Botrytis cinerea, Fusarium graminearum*, or *Ceratocystis pilifera* in test tubes at 27° C.

For identification the nematodes were fixed in heated TAF and processed by the rapid glycerol-ethanol method of Seinhorst (1959). Light microscope observations and drawings were made from fixed specimens.

Morphometric and allometric measurements were made on 40 females and 40 males of *B. mucronatus* extracted from wood samples of *Pinus koraiensis* Sieb. & Zucc. growing in the Pozarsky forest. The mean values for these parameters, the population standard deviations and the ranges for the various parameters were then calculated. Spicule lengths were measured along the median line of the arc of the spicule.

Maps of «The Atlas of the USSR» (Anonymous, 1986) were used to analyze the climate conditions in the Russian Far East. Mean daily temperature are shown in the atlas as isotherms at 4° C intervals for January and July.

RESULTS

The assays showed that the pinewood nematode, *B. xylophilus* was not present in any of the wood samples. However, the closely related species *B. mucronatus* Mamiya & Enda, 1979 was found (Table 1). The forest regions of the Primorsky territory that were surveyed and the sites where *B. mucronatus* was found are shown in Fig. 2.

Over 46% of the 230 wood samples contained nematodes of the orders *Tylenchida, Rhabditida* and *Aphelenchida*, but *B. mucronatus* occurred in only 13% of the samples. Only Scots and Korean pine from plantations in the Pozarsky, Kirovsky, and Tchernigovsky forests contained *B. mucronatus*. A Korean pine (*Pinus koraiensis* Sieb. & Zucc.) plantation at the Pozarsky forest was severely stressed and 12 of the 23 pines sampled yielded *B. mucronatus* nematodes. The maximum number of *B. mucronatus* in the trees was 20,000 specimens per 1 g of fresh wood.

Table 1. Results of nematode extractions from wood samples collected in the Primorsky territory of the Russian Far East.

Tree species	Samples assayed		
	Total number	With nematodes (%)*	With *B. mucronatus* (%)*
Pinus sylvestris L.	46	59	26
P. koraiensis Sieb. & Zucc.	106	38	16
Picea koraiensis Nakai.	20	60	0
P. ajanensis Fisch.	4	0	0
Larix daurica Turcz.	28	61	0
Abies nephrolepis Maxim.	26	35	0
Total	230	46	13

* The percent is calculated from the total number of samples.

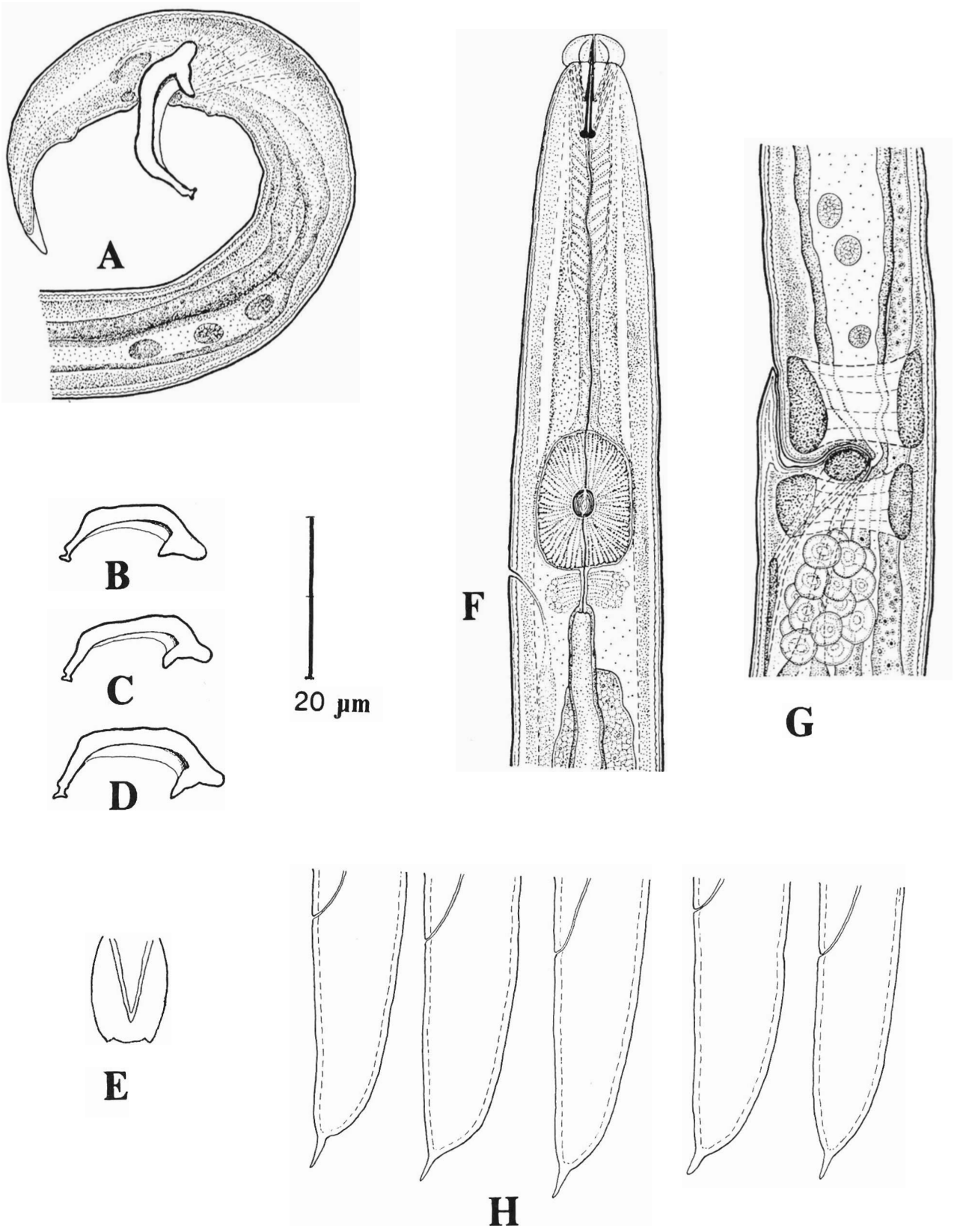

Fig. 1. *Bursaphelenchus mucronatus* (Primorsky isolate from the Russian Far East). Males. A: Posterior end; B - D: Spicules; E: Ventral view of tail tip with caudal alae. Females. F: Anterior end; G: Vulva region, lateral view; H: Tails.

The trees with these large *B. mucronatus* populations had died during the summer of 1993.

Also, during the survey 15 adult sawyer beetles (probably *Monochamus urussovi* Fisch.) were collected and assayed for nematodes. No nematodes were obtained from the beetles.

Specimens of *B. mucronatus* found during the survey were compared with the original description of *B. mucronatus* (Mamiya & Enda, 1979). However, similar comparisons have not yet been made with *B. mucronatus* from other localities, including those from within Russia. Based on the most recent research on the taxonomic relationships of various geographical isolates of *B. mucronatus*, and the suggestion that separate species status should be given to European and Japanese isolates (Abad et al., 1991; Beckenbach et al., 1992; Bolla & Boschert, 1993; Riga et al., 1991), our identification of the Primorsky forest nematodes as *B. mucronatus* is tentative. The measurements and a short description of the Primorsky *B. mucronatus* isolate are as follows:

Males (n = 40): L = 0.628 ± 0.074 (0.485-0.781) mm; a = 37.0 ± 4.8 (24.5-44.5); b = 10 ± 1.8 (6.6-13); c = 22.7 ± 3.7 (14.6-32.6); stylet = 13.2 ± 0.69 (12-14) μm; spicules = 23.1 ± 3.1 (18-31) μm. Body J shaped when killed by heat, lateral field not detected. Sperm round, 4-6 μm in diameter. Excretory pore opposite nerve ring (Fig. 1).

Females (n = 40): L = 0.84 ± 0.08 (0.68-0.99) mm; a = 38.8 ± 2.25 (34.8-44.3); b = 11.5 ± 0.9 (9.8-12.6); c = 24.8 ± 2.46 (20-30.7); V = 74.3 ± 1.54 (70.6-77.7) %; stylet = 13.5 ± 0.75 (12-15) μm. Body C shaped when heat-killed. Lateral field with four incisures. Vulva with cuticular flap. Post-uterine sac elongate, about seven times the body width at vulva. Tail conoid, with a mucro about 4-5 μm long (Fig. 1).

The Primorsky *B. mucronatus* differs from the original description of *B. mucronatus* from Japan (Mamiya & Enda, 1979), by having a shorter body and shorter male spicule, and by the shorter stylet in both males and females, i.e., in the Japanese isolate the parameters for the males are: L = 0.79 (0.64-0.97) mm, spicules 26 (23-29) μm, stylet = 15.0 (14-16) μm and the female stylet is 15.8 (14-16) μm. Also the Japanese *B. mucronatus* is characterized by the form of the mucro on the tail (Fig. 1).

DISCUSSION

This survey of conifer forests in the Russian Far East has failed to detect the pinewood nematode *B. xylophilus*. These data confirm the results of surveys by plant quarantine services of those countries which have imported the raw softwood products from Russia and earlier from the former USSR. According to those surveys only *B. mucronatus* occurs in timber from Russia (Braasch, 1991; Tomminen et al., 1989; Rutherford et al., 1990). The report from the Chinese Plant Quarantine Service which stated that the PWN was present in the logs imported from Russia (Anonymous, 1991) needs to be confirmed.

It has been suggested that the PWN was introduced into East Asia from North America (De Guiran & Brugier, 1989) and that the nematode has now spread to Japan, China and South Korea. Based on our results and the data from the plant quarantine services of other countries we believe that the PWN has not spread to the Russian Far East. This is despite the fact that all the prequisites exist for the PWN to become established there, i.e., the area contains susceptible hosts such as *Pinus sylvestris* L. and *P. koraiensis* Sieb. & Zucc., and suitable vectors (e.g. *Monochamus sutor* L. and *M. urussovi* Fisch.), and a favorable climate. In Japan, pine wilt disease is restricted to that part of the country where the August mean air temperature isotherm is 20° C or greater (Rutherford et al., 1990). The mean air temperature isotherms for the warmest summer month, i.e., July, for the Primorsky territory are show in Fig. 2. There the 20° C mean air temperature isotherm borders the region where pine wilt disease could occur. The size of this area could increase during hot summers.

The finding that *B. mucronatus* is widely distributed in the Primorsky territory is important. Previously *B. mucronatus* was found in Norway (McNamara & Stoen, 1988; Finland (Tomminen et al., 1989), Sweden (Magnusson & Schroeder, 1989), France (Baujard et al., 1979), Italy (Palmisano et al., 1992), Japan (Mamiya & Enda, 1979), China (Cheng

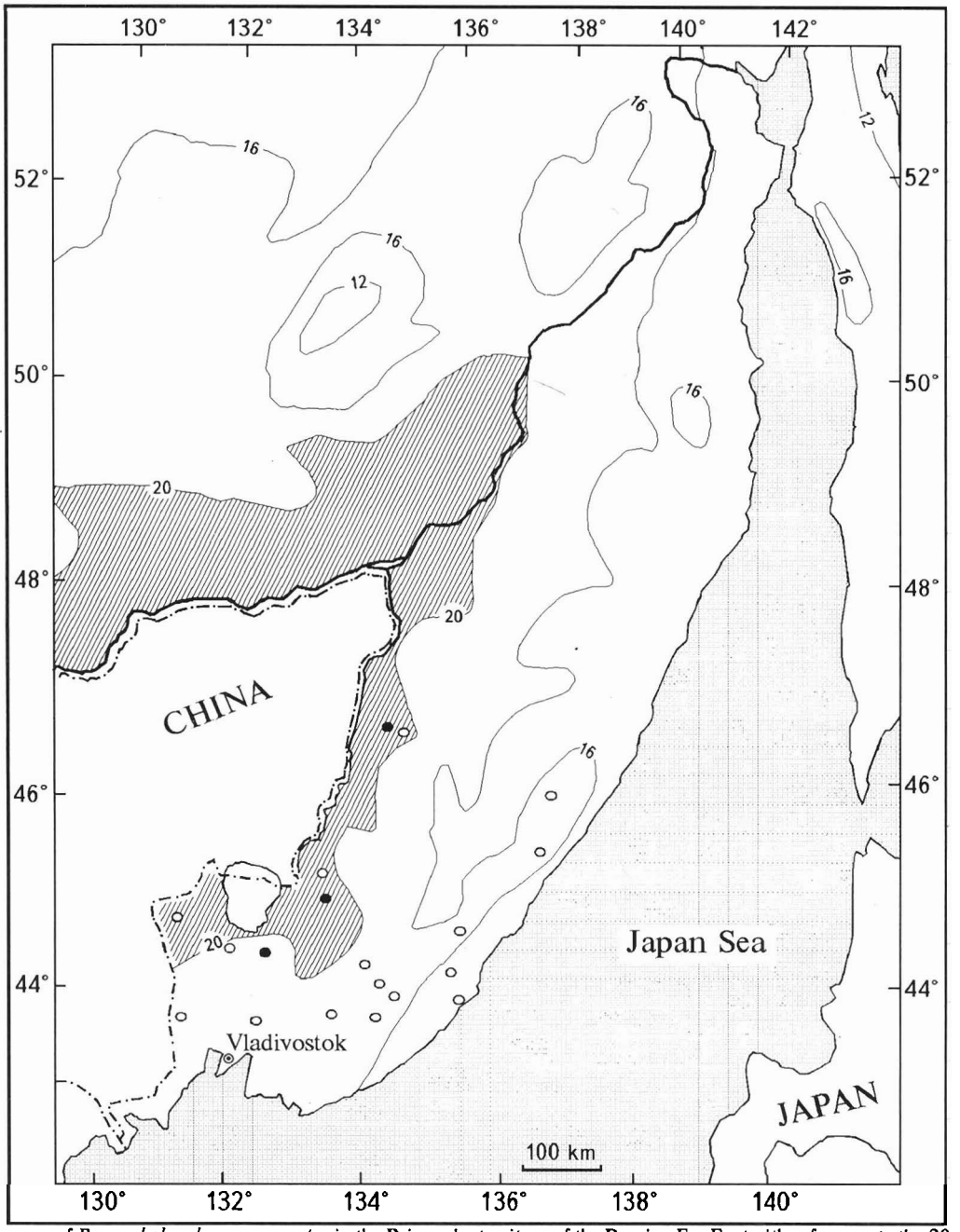

Fig. 2. Occurrence of *Bursaphelenchus mucronatus* in the Primorsky territory of the Russian Far East with reference to the 20 and 16 July mean air temperature isotherms. (● - *B. mucronatus* present; o - *B. mucronatus* absent finding; ▨ - area where climate is suitable for PWN

et al., 1986), South Korea (Choi & Moon, 1989) and Russia. Inoculation experiments showed that while *B. mucronatus* is pathogenic on conifers, it is less virulent than *B. xylophilus* (Bakke et al., 1991; Futai, 1985; Mamiya & Enda, 1979; Schauer-Blume, 1990). European *B. mucronatus* isolates are considered to be more pathogenic than Japanese isolates (Riga et al., 1991). Also, it has been shown that by mating *B. mucronatus* and *B. xylophilus* it is possible to produce hybrids that are as pathogenic as the *B. xylophilus* parental isolates and more pathogenic than the *B. mucronatus* parents (Riga et al., 1991).

Reports on the pathogenicity of *B. mucronatus* (Schauer-Blume, 1990; Bakke et al., 1991) and the fact that large populations of *B. mucronatus* nematodes were present in Korean pines which died during the

summer in the Pozarsky forest, suggest that *B. mucronatus* could be the primary cause of tree death. Tests need to be made to determine the pathogenicity and host range of the Primorsky *B. mucronatus* isolates.

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Кулинич О.А., Круглик И.А., Ерошенко А.С., Колосова Н.В. Обнаружение и распространение нематоды *Bursaphelenchus mucronatus* на Дальнем Востоке России.

Резюме. В летние сезоны 1991 - 1993 гг. проводились обследования лесных массивов на территории Приморского края России - районе наиболее вероятного обнаружения опасного карантинного паразита хвойных пород сосновой стволовой нематоды *Bursaphelenchus xylophilus*. Анализировались ветки и спилы со стволовой части погибших и погибающих деревьев сосны (*Pinus sylvestris* L., *P. koraiensis* Sieb. & Zucc.), ели (*Picea koraiensis* Nakai, *P. ajanensis* Fisch.), лиственницы (*Larix dahurica* Turcz.) и пихты (*Abies nephrolepis* Maxim.). *B. xylophilus* в пробах не обнаружен, в то же время установлено широкое распространение на территории Приморского края близкого к нему вида *B. mucronatus*. Даны морфологическое описание и рисунки данного изолята. *B. mucronatus* обнаружен в древесине сосны обыкновенной (*P. sylvestris*) и сосны корейской (*P. koraiensis*). При этом максимальная численность особей достигала 20000 особей в 1 г древесины сосны корейской. Большинство зараженных нематодами деревьев погибли в течение одного летнего сезона. Это дает основание предполагать, что нематоды могли быть первопричиной их гибели.