



# Host and vector surveys for the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Nematoda: Aphelenchoididae) in Canada

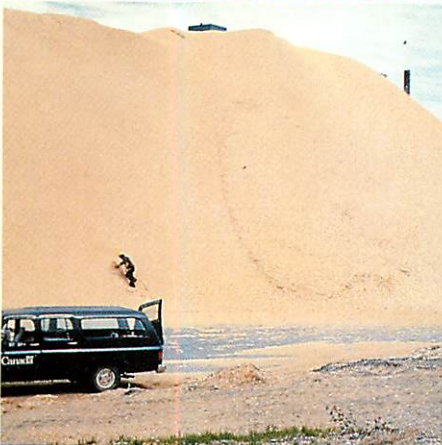
W.W. Bowers, J. Hudak and A.G. Raske (Editors)

Newfoundland and Labrador Region • Information Report N-X-285

with contributions from:

L.P. Magasi, Maritimes Region • D. Lachance, Quebec Region • D.T. Myren, Ontario Region

H.F. Cerezke, Northwest Region • G.A. Van Sickle, Pacific and Yukon Region



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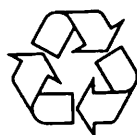
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- E. Sampling wood borer-infested logs for pinewood nematode.
- F. Tail section of pinewood nematode (male).



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**HOST AND VECTOR SURVEYS FOR THE PINEWOOD NEMATODE,  
*BURSAPHELENCHUS XYLOPHILUS* (STEINER AND BUHRER)  
NICKLE (NEMATODA: APHELENCHOIDIDAE) IN CANADA**

**Forestry Canada**  
Forest Insect and Disease Survey

W.W. Bowers, J. Hudak and A.G. Raske (Editors)  
**Newfoundland and Labrador Region**

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Information Report N-X-285  
1992

Forestry Canada  
Newfoundland and Labrador Region

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ISSN NO. 0704-7657  
CAT. NO. Fo46-15/285E  
ISBN NO. 0-662-19919-7

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The pinewood nematode occurs in low numbers in six species of pines in Canada: Scots pine, jack pine, lodgepole pine, red pine, Ponderosa pine and eastern white pine. The pinewood nematode is also present in low numbers in six other common conifers: balsam fir; white spruce, black spruce, red spruce; tamarack and Douglas-fir. The nematode is absent in hemlock and the various species of cedars. Almost all trees infested by the nematode were weakened by other factors.

A total of 5619 insects, including 1294 *Monochamus* sp., were examined for the presence of pinewood nematode. The nematode was isolated from only one specimen of *Monochamus clamator* collected in the field. No other potential vectors contained *B. xylophilus*, including those vectors that had emerged from logs artificially inoculated with high numbers of the nematode.

The risk of importing the pinewood nematode with Canadian lumber and logs, or indirectly through vectors, is considered extremely low, especially with continued programs to eliminate bark and grub holes. Furthermore, the high threshold temperatures (20°C+) required for the development of pine wilt disease would preclude disease expression in much of Europe. Moreover, mucronated forms of *Bursaphelenchus* are known to occur in France, Norway, Finland, Sweden and Germany.

De petites quantités de nématodes des pins se présentent sur six espèces de pin examinées au Canada : le pin sylvestre, le pin gris, le pin de Murray, le pin rouge, le pin ponderosa et le pin blanc. De petites quantités de nématodes des pins se présentent également sur six autres conifères communs : le sapin baumier, l'épinette blanche, l'épinette noire, l'épinette rouge, le mélèze et le Douglas taxifolié. Le nématode est absent de la pruche et des diverses espèces de cèdre. Presque tous les arbres affectés par le nématode étaient affaiblis par d'autres facteurs.

Un total de 5 619 insectes, dont 1 294 du genre *Monochamus*, ont été examinés pour déterminer la présence de nématodes des pins. Le nématode n'a été isolé que sur un spécimen de *Monochamus clamator* recueilli sur le terrain. Aucun autre vecteur potentiel ne portait *B. xylophilus*, même ceux qui avaient émergé de rondins inoculés artificiellement avec de grandes quantités du nématode.

On considère que le risque d'importer le nématode des pins avec du bois d'oeuvre et des rondins canadiens, ou indirectement par des vecteurs, est extrêmement faible, particulièrement si l'on mène régulièrement des programmes pour éliminer les trous dans l'écorce et les galeries d'insectes. En outre, le seuil de température élevé (20° C et plus) nécessaire à l'incubation de la maladie de flétrissement du pin suffit à exclure l'apparition de cette maladie dans une grande partie de l'Europe. Qui plus est, on reconnaît déjà la présence de formes mucronées du *Bursaphelenchus* en France, en Norvège, en Finlande, en Suède et en Allemagne.

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## ACKNOWLEDGMENTS

We thank the field staff of the Forest Insect and Disease Survey in the regional centres of Forestry Canada for their efforts in collecting samples of trees and potential vectors. We also thank the laboratory staff in these regions for technical assistance. F.M. Ring of the Pacific and Yukon Region, C.N. Davis and B. Smitt of the Ontario Region and K.J. Harrison of the Maritimes Region took on extra responsibilities in the pinewood nematode survey for which we are grateful. We thank E.S. Kondo (Forest Pest Management Institute, Sault Ste. Marie ON), L.W. Carlson, B.H. Moody and P. Singh (Forestry Canada, Ottawa ON), G.M. Howse (Forestry Canada, Sault Ste. Marie ON) for assistance in planning and encouragement throughout the survey. We are indebted to R.V. Anderson and B. Ebsary, Research Branch, Agriculture Canada, J. Scott and R. Favrin, Plant and Animal Health Directorate Agriculture Canada, Ottawa ON and to J.R. Finney-Crawley, Department of Biology, Memorial University of Newfoundland, for extraction and identification of nematodes. Members of the Maritimes Lumber Bureau cooperated with sampling logs and lumber in their mill yards. We also acknowledge the cooperation of the Council of Forest Industries of British Columbia (COFI). We are grateful to the staff of the Newfoundland Department of Forestry and Agriculture for collecting tree samples in Labrador. We thank R.J. West, D.M. Stone, J.E. Farrell and L.R. Larose (Forestry Canada, Newfoundland and Labrador Region) for critical review of the manuscript, preparing the maps, providing technical assistance and typing the manuscript, respectively.

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**INTRODUCTION**

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhner) Nickle (= *B. lignicolus* Mamiya and Kiyohara) is an important forest pest because it causes wilting and considerable tree mortality, particularly in Japan. The nematode also is a major impediment to international trade of forest products (Dwinell and Nickle, 1989).

*B. xylophilus* has caused sudden wilting and extensive mortality of pines in Japan and has been intensively studied since the late 1960's (Kondo *et al.* 1982; Kobayashi *et al.* 1984; Mamiya 1983, 1984, 1988).

The pinewood nematode was first associated with tree mortality in North America in the United States in 1979 (Dropkin and Foudin 1979). The nematode was associated with dying trees of several species of pine, mainly exotic ornamentals (Dropkin *et al.* 1981; Bergdahl 1982, 1988; Mamiya 1984). In 1982, *B. xylophilus* was reported on jack pine from southern Manitoba, Canada (Knowles *et al.* 1983). However, there are no reported cases of tree mortality

caused by the pinewood nematode in Canada.

The European Plant Protection Organization (EPPO) placed *B. xylophilus* on the A1 list of quarantine pests in 1986 (Anon. 1986). The decision to rank the pinewood nematode as a potentially important pest and a concomitant ruling by Scandinavian countries to restrict the importation of conifer chips and timber from countries where the pinewood nematode is known to occur is a major concern of Canadian forest managers and lumber exporters. An improved understanding of the incidence and behaviour of *B. xylophilus* is needed to address the concerns of EPPO and Canadian lumber exporters. Furthermore, assessment of the level of risk associated with pinewood nematode is contingent on identification of potential vector(s) responsible for the transmission of pinewood nematode.

The Forest Insect and Disease Survey (FIDS) of Forestry Canada initiated a nationwide survey in 1985 to determine the distribution of the pinewood nematode. This report reviews briefly the history and biology of the pinewood nematode and presents the results of the Canadian survey.

## REVIEW OF PINEWOOD NEMATODE

### History

*B. xylophilus* has been reported in the United States, Canada, mainland China, Taiwan, and Japan (Mamiya 1988). In Asia the nematode causes wilting and considerable tree mortality, however, in North America *B. xylophilus* is not associated with epidemic disease (Wingfield *et al.* 1984; Dwinell and Nickle 1989).

Pine wilt symptoms accompanied by tree mortality were first recorded in Japan in 1905 (Mamiya 1983), but *B. xylophilus* was identified as the causal agent only in 1969 (Tokushige and Kiyohara 1969; Kiyohara and Tokushige 1971). Three years later, members of the long-horned wood borer genus *Monochamus* (Coleoptera: Cerambycidae), were identified as the vectors responsible for transporting the nematode among trees (Mamiya and Enda 1972).

*B. xylophilus* was first isolated in 1929 from trees in the United States (Steiner and Buhner 1934), but was not recognized as the causal organism of pine wilt disease until 1979 (Dropkin and Foudin 1979). Subsequently, surveys in the United States recorded the nematode in 34 of the 48 contiguous states of the United States (Robbins 1982; Bedker *et al.* 1984; Kinn 1986a). Tree mortality attributed to pine wilt disease has been limited to areas of warm climates of central and southern United States (Wingfield *et al.* 1982; Rutherford and Webster 1987;

Rutherford, *et al.* 1990). Vectors of the pinewood nematode in the United States are members of the cerambycid genus *Monochamus* (Linit *et al.* 1983; Wingfield 1983).

In Canada, surveys to determine the presence and status of the pinewood nematode were initiated in 1980 (Sterner and Davidson 1982, 1983; Moody and Cerezke 1984; Van Sickle 1991). In 1982, the nematode was reported from Manitoba (Knowles *et al.* 1983) and its presence was confirmed by Forest Insect and Disease Survey in 1983 (Kondo and Taylor 1984). A coordinated national survey for the pinewood nematode and its vector in Canada was initiated in 1985. The nematode was detected in 1985 in Ontario (Juzwik and Myren 1986; Kondo and Taylor 1986), Quebec (Lachance 1987), New Brunswick and Nova Scotia (Magasi 1987), and in British Columbia in 1986 (Wood and Van Sickle 1987). In 1987, the nematode was reported in Newfoundland (Clarke and Carew 1988), Saskatchewan and Alberta (Moody 1988).

The European Plant Protection Organization (EPPO) concluded that the pinewood nematode meets all the criteria of a class "A-1" pest (=potentially important) in 1986 (Anon. 1986). Finland, in 1985, restricted the importation of conifer chips and timber from countries where the pinewood nematode is present because wood chips shipped from the United States and Canada in 1984 were infested by the nematode (Rautapää 1986). The other Scandinavian countries acted similarly and EPPO recommended that Europe ban softwood products except kiln-dried lumber from countries with

pinewood nematode (Magnusson 1986; Venn 1986; McNamara and Stoen 1988).

## Biology

### Pinewood Nematode

Adult pinewood nematodes occur in the wood of twigs and boles of host trees where they mate and the female produces up to 80 eggs within 28 days (Mamiya and Furukawa 1977). The nematode can complete its life cycle in four to five days under favorable conditions and warm temperatures (Mamiya 1984). The first larval stage is passed within the egg (Holdeman 1980) and the second stage hatches but soon molts to the third stage. The third larval stage can exist in two forms; the expected propagative stage that yields adults that stay in the tree, or a resistant stage suitable for transport to other trees. Factors that enable the resistant third larval stage to withstand starvation, low temperatures and dry conditions include a thickened cuticular layer and densely packed lipid droplets in their bodies (Mamiya 1984). Third stage larvae molt to the fourth stage resistant dauerlarvae which infest the vector in the xylem of trees. The vectors of the pinewood nematode are beetles of the long-horned wood borer genus *Monochamus* (Mamiya 1984; Garland 1985). The insect vectors emerge from host trees, fly to new trees, and feed on the thin bark and phloem of twigs. Dauerlarvae of the nematode leave the vector at this time and enter the host tree via feeding-wounds caused by the vector, and feed and molt into the adult stage. Thus the life cycle of the nematode

involves six life stages: egg, four larval stages and the adult.

Adult and larval nematodes feed on the epithelial cells and resin ducts of susceptible trees (Dwinell and Nickle 1989), and may also feed on various fungi within the trees (Wingfield 1987; Myers 1988). The initial stress within trees may result from toxins released by the nematode (Oku *et al.* 1979) or by the associated bacteria (Kondo *et al.* 1982). Four toxic compounds that apparently act synergistically have been identified (Bolla *et al.* 1982; Oku 1988). *B. xylophilus* can multiply rapidly in stressed trees and destroy the resin canals, epithelial and ray parenchyma cells. The death of several cell types, the production of toxin, and the leakage of oleoresin and other substances into the tracheids greatly reduces the water potential in the roots and needles (Melakeberhan *et al.* 1991), and cause the death of pines within weeks of infection (Myers 1988). Unlike many other nematodes, the pinewood nematode cannot survive long in the soil (Mamiya and Shoji 1988), and transmission from tree to tree through the soil has not been demonstrated (Dwinell and Nickle 1989).

### *Monochamus* Vector

*B. xylophilus* is closely associated with *Monochamus* spp., and is primarily dependent upon members of this genus for transport to suitable hosts (Mamiya 1984; Garland 1985; Shibata 1985; Togashi 1985, 1988; Shibata and Okuda 1989). Adults of *Monochamus* spp. emerge from host trees and fly to the crown of adjacent trees to feed on the bark and phloem of twigs during their maturation period.

Subsequently, both sexes attack weakened trees or recently cut logs on which to mate and lay eggs. Larvae hatch within a week from eggs, feed on the phloem, and by fall, tunnel into the xylem to form galleries. The larval galleries are enlarged to form the pupal chamber when the larvae are fully grown. Larvae overwinter and pupation occurs the following spring. The beetles complete the larval stages within one year in warmer climates, or in logs exposed to full sunlight, but may take two or three years in regions of cooler climate. Adults emerge in the summer after pupation (Rose 1957; Raske 1973; Cerezke 1977).

*B. xylophilus* is attracted to *Monochamus* pupae by the increased concentration of carbon dioxide within the pupal cell (Miyazaki *et al.* 1978). The nematodes invade the pupae, and tend to congregate, in numbers as high as 289 000, within the spiracles and tracheae of the insect (Linit 1987, 1988). However, vectors emerging from trees killed by the nematode usually carry only several thousand nematodes (Linit 1988). The adults are not noticeably hindered during flight by large numbers of nematodes they may carry (Humphry and Linit 1989).

The transmission of the nematode to new hosts through feeding wounds on twigs is called "primary" (Linit 1987), because this infection may result in the primary cause of stress or mortality of susceptible tree species. However, the tree may also be infested with the nematode through oviposition niches established by the female (Wingfield and Blanchette 1983; Luzzi *et al.* 1984). Transmission by females that oviposit into predisposed trees is termed "secondary" transmission, and

implies that the nematode is not the primary cause of reduced tree vigor.

### Host-Vector-Nematode Interaction

*B. xylophilus* and its *Monochamus* vector are mutually beneficial on susceptible host trees in areas where pine wilt disease occurs. The nematode is dependent on its vector for transport to a new host tree, where it will complete its life cycle. In a susceptible host the nematode may increase to large numbers killing the trees by feeding and by release of toxins. After two to three years a dead tree becomes unsuitable and the nematode depends on a vector to carry it to new hosts. The nematode may persist in non-susceptible trees, but it remains at very low population levels (Mamiya 1984; Dwinell and Nickle 1989).

*Monochamus* selects only weakened or dying trees for oviposition and larval development (Rose 1957; Cerezke 1977). Healthy trees appear to resist beetle establishment by producing large quantities of oleoresin toxic to eggs or young larvae. The introduction of the pathogenic nematode to healthy trees during maturation feeding lowers host resistance and will facilitate beetle establishment.

### Taxonomy

*B. xylophilus* belongs to a complex of species whose exact relationship to each other is not fully understood. Morphological comparisons, cross-breeding experiments and recent use

of molecular probes have provided most of the evidence for taxonomic designations.

The virulent forms of *B. xylophilus* have a round tail and cause pine wilt disease in the warmer parts of Japan, China and the United States (Nickle *et al.* 1981). Another species, *B. mucronatus* was described from Japan (Mamiya and Enda 1979) as having a mucronate tail (females only) and not causing any stress to pines in Japan (Tamura and Enda 1986). Cross-breeding and other experiments demonstrated that the two species do not interbreed, and are morphologically and molecularly distinct (Mamiya 1986; Riga and Webster 1989). Presently two forms of *B. xylophilus* are recognized in North America; the "r", or round-tail form (= non-mucronate), and the "m" form whose females have a mucro on the tail. In North America the "r" form most always occurs in pine, and the "m" form occurs most often in fir and spruce, though it may also occur in pine and in other conifers (Wingfield *et al.* 1983; Dwinell and Nickle 1989; Abad *et al.* 1991).

Paradoxically, the "m" form that occurs in central North America, the "Minnesota strain", (morphologically identical to *B. mucronatus* of Japan) has closer affinities to *B. xylophilus* than it does to *B. mucronatus* of Japan. The North American "r" and "m" forms have similar mating potential, identical chromosome complements, overlapping geographic ranges, and in greenhouse studies have similar virulence (Bolla and Boschert 1990; Sutherland *et al.* 1991). However, the "Minnesota strain" differs molecularly from the virulent strains of *B. xylophilus* (Abad *et al.* 1991).

Two or three other species, of about 50 species of *Bursaphelenchus* that inhabit trees, are also closely related to *B. xylophilus*. The French and Norwegian isolates of *B. mucronatus* are of greatest interest. Both of these isolates have closer affinities to the Japanese *B. mucronatus*, than to the North American "m" form (Abad *et al.* 1991). Crossing the French isolate of *B. mucronatus* with Japanese isolates of both *B. xylophilus* and *B. mucronatus* produced viable offsprings (de Guiran and Boulbria 1986; Webster *et al.* 1990), indicating close genetic affinity of the French isolate to both Japanese "species". However, the offspring of crosses of French and North American isolates have reduced viability (Hajdukiewicz and Myers 1988; de Guiran and Bruguier 1989), indicating more distant genetic affinities. Webster *et al.* (1990), using DNA probes, confirmed the closer affinity of the French *B. mucronatus* to the Japanese isolates than to the North American isolates. Recently, Tares *et al.* (1992) investigated the relationships between isolates of pinewood nematode using homologous DNA probes and confirmed the existence of a *B. xylophilus* group and a *B. mucronatus* group. For the *B. xylophilus* group, three geographical subgroups were identified: the United States, Canada and Japan with a close relationship between USA and Japanese isolates. Furthermore, hybridization patterns of the Minnesota isolate found on *Abies balsamea* indicated that it belongs to *B. xylophilus* even though it possesses a mucronated tail characteristic of *B. mucronatus*.

Species and geographical isolates of *Bursaphelenchus* with the exception of *B. fraudulentus*, Rhüm, do not appear to

be reproductively isolated. Therefore, it is appropriate to consider the three species (*B. xylophilus*, *B. mucronatus*, and *B. fraudulentus*) as members of a *supraspecies* with pathogenic and non-pathogenic isolates in the complex. Members can be characterized by common traits: 1. all virulent isolates have rounded tails ("r" form), and; 2. the mucronate isolates ("m" form) are not pathogenic in unstressed pine or other conifers in natural conditions. The general picture is obscured because some mucronate isolates have individuals with very small mucros or none at all (Fukushige and Futai 1985; Webster *et al.* 1990), and some mucronate isolates cause wilting and mortality of seedlings in high-temperature greenhouse conditions (Bakke *et al.* 1991; Sutherland *et al.* 1991).

### Pathogenicity

Mortality in the field has occurred in five major species of pine known to be susceptible to the pinewood nematode: Scots pine, Japanese red pine, Japanese black pine, Armand pine and slash pine. Slash pine is the most resistant among these five species. These species of pine are susceptible when growing in areas where high temperatures consistently exceed approximately 20°C for several weeks (Rutherford and Webster 1987). In addition, pines that are normally resistant, such as red pine, may become susceptible when planted in areas of warmer temperatures than occur in their natural range; even though the temperatures may not reach the extremes of more than 20°C for long periods (Rutherford *et al.* 1990).

Considerable knowledge has accumulated concerning the pathogenicity of the pinewood nematode from seedling inoculations in greenhouses. Many seedlings of pines, spruce, fir, and larch were killed by *B. xylophilus* at high temperatures in greenhouse experiments in Canada, the United States and Europe. In contrast, yellow cypress, eastern white cedar, western red cedar and western hemlock were demonstrated to be resistant to pinewood nematode (Mamiya 1983; Futai and Sutherland 1989; Panesar and Sutherland 1989; Schauer-Blume 1990; Bakke *et al.* 1991; Riga *et al.* 1991; and Sutherland *et al.* 1991).

The degree of susceptibility of seedlings to the nematode in greenhouses was directly related to the temperature (Panesar and Sutherland 1989; Schauer-Blume 1990; Bakke *et al.* 1991; and Sutherland *et al.* 1991). More tree species were susceptible at temperatures above 25°C and seedlings showed symptoms earlier and died faster.

### Control

Numerous control tactics have been investigated, but no effective management strategy for *B. xylophilus* exists. Wood-dwelling insects and nematodes can be rapidly killed by high temperatures (Dwinell 1986, 1990; Kinn 1986a, 1986b; Tomminen and Nuorteva 1992), therefore, kiln-drying or other heat-treatment offers high potential as a control tactic. Recent studies on the use of heat-treatment provided effective and practical pasteurizing schedules for the eradication of pinewood nematode and its vectors from



unseasoned softwood lumber in Canada. Experiments established that a core temperature of 59°C for 30 minutes is required to effectively pasteurize lumber regardless of species, specific gravity, moisture content and thickness. The successful use of pasteurization was demonstrated at three different locations in Canada using conventional, high temperature and dehumidification kilns (Smith 1991). Similar studies were conducted by the European Community (Evertsen 1991).

Wood chips may be treated for nematodes in transit, and washing chips in a 15 % solution of sodium N-methyldithiocarbamate or in hot water is effective (Kinn and Springer 1985; Kinn and Deister 1987). The use of steam heating and fumigants (such as methyl bromide and phosphine) has been investigated (Anon. 1990) but these are presently impractical or prohibited by pesticide regulations. Research on alternative methods, such as breeding trees for resistance, biological control, the use of microwaves, irradiation and ultrasonification is in progress (Saiki *et al.* 1984; Fukuda *et al.* 1989; Anon. 1990).

## **SURVEY METHODS**

### **Sampling Potential Host Trees**

A national survey for pinewood nematode was initiated by Forestry Canada in 1985. Trees sampled in the survey included: 1. the major forest conifer species of each Region, and; 2. the native

and exotic tree species most likely to harbor the nematode, particularly those trees that were dead or dying which represented the highest risk to infection and the worst-case scenario.

The following priority of tree species was established:

- Priority 1      Hard pines in all regions of Canada.
- Priority 2      True firs, spruces and soft pines in all regions of Canada.
- Priority 3      Douglas-fir and larches wherever they occur.
- Priority 4      Other conifers in all regions of Canada.

The host surveys were intensified from 1986 to 1988 (Kondo and Taylor 1986; Kondo and Moody 1987; Moody 1988, 1989; Magasi and Harrison 1990).

Destructive and non-destructive sampling methods were used to sample for *Bursaphelenchus*. One 5 cm-thick disk was cut from trees at 1.3 m above ground, near mid-bole, and in the top third of the bole. Two disks were collected from small trees; one from 1.3 m above ground and the other from the lower limit of the live crown. Logs were sampled by removing large chips with an axe. Trees were also sampled by collecting shavings from borings with a drill and bit at 0.5 m, 1.3 m and 2.0 m above ground. A bit of 40 mm diameter was used and the shavings from a hole 5 cm deep collected after the bark and phloem had been removed. All equipment was cleaned and surface

sterilized with 70% alcohol between collection of samples.

Disks, chips and wood shavings were placed in plastic bags, with moist paper towels, sent to the laboratory within 48 hours, and stored at 4°C until 24 hours before processing at room temperatures. Wood samples were cut into small chips, wrapped in cheese cloth or chem-wipe tissue, submerged in water in a Baermann funnel, and kept for 24 to 48 hrs. Water was tapped from the funnel and examined for the presence of nematodes. All wood samples negative for the presence of nematodes were left at room temperature for a further four days and reprocessed. Adult nematodes were identified and juveniles were placed on potato dextrose agar plates with the fungus *Botrytis cinerea* for rearing to the adult stage. Permanent slides of adult nematodes were prepared by killing the nematodes in hot water, fixing in F.A. 4:1 (formalin : acetic acid) for at least 24 hr, and processed using Seinhorsts method and mounted in glycerine (Finney-Crawley 1989a).

### Sampling Potential Insect Vectors

Standardized methods for collecting and processing insect vectors were developed in 1987 and used in all regions of Canada (Finney-Crawley 1989b). Sampling methods were based on a set of priorities including type of material and insect species as follows:

#### Type of Material

Priority 1 Wood borers in the host, recently emerged wood

borers from infested material, and wood borers and bark beetles excised from the host.

Priority 2 Insects attracted to wood chip piles. This collection site had high priority because of the importance of wood chips to the export market.

Priority 3 Insects landing on host trees and wood piles.

Priority 4 Wood borers collected from flowers, or other non-host or miscellaneous sites.

### Insect Species

*Monochamus* spp. were considered potential vectors in Canada because they are the primary vectors of the pinewood nematode in Japan and the United States (Mamiya 1984; Linit 1988). Other wood- and bark-inhabiting insects that may be vectors of the nematode were also collected. The following priority was established to sample potential insect vectors (for more detail see Appendix 2):

Priority 1 *Monochamus* spp.

Priority 2 Other wood borers and primary bark beetles.

Priority 3 Secondary bark beetles.

Priority 4 Other wood- and bark-inhabiting insects.

### Collection of Insect Vectors

Logged areas and recently piled logs were preferred for collecting log-sections for rearing and for excising wood borers and bark beetles. This material was most likely to produce the greatest numbers of potential vectors if collected in May or early June, depending upon latitude and elevation. Insects at chip piles were hand-collected, netted or captured with the use of barrier traps.

Small log piles consisting of recently felled trees were also used to bait potential vectors. Log piles were established in spring to attract potential vectors to different species of trees. Multiple funnel traps (Lindgren 1983) were erected to intercept insects responding to logs piles.

### Handling, Storing and Shipping of Insect Vectors

Captured insects were kept alive until nematodes were extracted. Wood borers, especially *Monochamus* spp., were placed singly into vials with moistened tissue paper, but small bark beetles were placed in one vial with sufficient tissue paper, and at times with small particles of host materials to provide resting places. Vials were kept out of direct sunlight in the field and placed into cold storage the same day. All insects were shipped, within seven days, to J. Finney-Crawley, Memorial University of Newfoundland for extraction and identification of nematodes.

Depending on the type and abundance of material one of two methods were used to isolate nematodes: 1. Individual insects were examined externally in water.

Particular emphasis was placed on detection and extraction of nematodes from the spiracles, antennae, legs and under the elytra. An internal dissection of each insect was carried out in Lum's Ringer. 2. Insects were cut into small pieces, wrapped in cheese cloth and nematodes extracted for 24 hr at  $24 \pm 1^\circ\text{C}$  using the Baermann funnel technique. The second method was used at peak periods of insect collection. Nematodes from insect vectors were identified as described for host samples.

### Mill and Other Surveys

Eastern hemlock logs at 22 mill sites and hemlock lumber at two of these sites were sampled in New Brunswick and Nova Scotia (Magasi *et al.* 1990). A total of 1766 logs from trees felled in spring and early summer were examined for wood borer oviposition. A total of 167 uninfested and wood borer-infested logs were sampled for nematodes. In addition, 188 piles of lumber were surveyed by examining at least 20 boards, or sufficient boards to find grub holes. A total of 28 samples were collected from uninfested and cerambycid-infested lumber.

Additional sampling for pinewood nematode in British Columbia in 1990 emphasized western hemlock and western red cedar. A total of 19 log yards and 30 mill sites were inspected and more than 100 logs or boards were sampled in each with attention focused on older insect-infested logs and low grade boards (Wood and Van Sickle 1991). In addition, 96 wood borer-infested logs of spruce, lodgepole pine and Douglas fir were

artificially inoculated with high numbers of the pinewood nematode and the 1762 insects that emerged from these bolts were checked for the nematode.

Staff of the Newfoundland Department of Forestry and Agriculture sampled a total of 94 locations near Goose Bay, Labrador, primarily in or near proposed commercial logging operations. A total of 462 trees were sampled consisting of 223 balsam fir, 224 black spruce, four white spruce and 11 larch. All trees sampled were either stressed, dying or had died within a year, which maximized the probability of the presence of the pinewood nematode. Trees were sampled from late November 1991 to late February 1992. This survey and its results are included in this report because methods and objectives were similar to the national survey from 1985 to 1990.

## **SURVEY RESULTS**

### **Host Tree Surveys**

The Forest Insect and Disease Survey sampled a total of 3706 trees from 1985 to 1990 to determine the presence of the pinewood nematode in Canada. This sample consisted of 1309 pines, 833 firs, 561 spruces, 123 Douglas fir, 221 cedars, 192 western hemlock, 107 larches and 40 trees of other species (Table 1). All major forest conifer species of Canada and several exotic species were sampled. Approximately 25% of the trees were healthy, 20% were damaged by insects or

diseases or were dying, and about 55% had died recently.

The pinewood nematode, both the "m" and "r" forms, occurred in small isolated groups of one or two trees at 277 locations in Canada (Table 2, Figs. 1-6). The number of trees containing the pinewood nematode was 330 of the total of 3706 sampled (Tables 1, 2). More than one tree species or more than one tree of the same species were sampled at some locations, therefore, the number of trees with pinewood nematode exceeded the number of locations.

Incidence of pinewood nematode was the highest in Ontario and Quebec where the nematode was present at 180 and 47 locations respectively (Table 2, Figs. 3, 4). The number of locations with positive samples in western and eastern Canada was lower than in central Canada. The three Prairie Provinces had positive samples at nine locations and British Columbia only at six. The pinewood nematode was not isolated on Vancouver Island in British Columbia. The frequency of occurrence in Atlantic Canada was also low in each province. The number of locations with positive samples was 18 in New Brunswick, nine in Nova Scotia and eight in Newfoundland. The pinewood nematode was not isolated in Prince Edward Island.

The frequency of the pinewood nematode in relation to the number of trees sampled in each province, was also highest in Ontario and Quebec, where 221 of 642 trees and 48 of 340 trees sampled, respectively, were positive for the pinewood nematode (Tables 1, 2). The corresponding figures for the other

provinces were: 21 of 238 trees for New Brunswick; 11 of 135 trees for Nova Scotia; 14 of 524 trees for Newfoundland; only six of 1298 for British Columbia; and, 0 of 55 for Prince Edward Island.

A total of 2773 of the 3706 trees sampled were either dying or dead (Table 1), representing a worst-case scenario in sampling for the presence of the pinewood nematode. Only 6% (44 of 748) and 14% (277 of 2025) of the dying and dead trees, respectively, were sources of the pinewood nematode in Canada. Less than 1% (9 of 933) of the healthy trees sampled yielded pinewood nematode isolates (Tables 1, 2).

The "r" form of the nematode was isolated from a total of 92 trees in Canada, including seven from New Brunswick, 14 from Quebec, 66 from Ontario, three from Manitoba and two from Alberta (Table 2). The "r" form was not isolated in Nova Scotia in this tree survey, but inspection of lumber samples confirmed the presence of this form. The occurrence of the "r" form was limited to pine except in Ontario where it occurred in three samples of balsam fir and in two samples of white spruce.

The "m" form of the pinewood nematode occurred in all provinces of Canada except in Prince Edward Island. The "m" form was isolated in a total of 132 samples, and was far more common in fir and spruce trees (95) than in pine (32) (Table 2).

The pinewood nematode was isolated from 12 tree species in Canada (Tables 1, 2): Scots pine, jack pine, lodgepole pine, red pine, Ponderosa pine, and eastern white pine, balsam fir, white spruce, black

spruce and red spruce, tamarack and Douglas-fir. Generally, the nematode occurred in dead or dying trees but it was also isolated from nine healthy trees: tamarack, five trees; balsam fir, black spruce, jack pine and eastern white pine, one tree each (Table 2).

In addition to *Bursaphelenchus*, numerous other genera of nematodes were isolated from healthy, moribund and dead trees (Table 4).

### Vector Surveys

The survey of vectors emphasized the collection of *Monochamus* spp., other long-horned wood borers (= Cerambycidae, round-headed borers), metallic wood borers (= Buprestidae, flat-headed wood borers) and other beetles (Coleoptera) in descending order of priority (Table 3, App. 2). Populations of *Monochamus* were generally low in Newfoundland and Nova Scotia and only 18 and 24 individuals of these beetles were collected in these provinces respectively. However, more than 250 specimens of *Monochamus* were collected in each of New Brunswick, Quebec, Ontario and British Columbia. Five species of the genus were identified among the collections, but the majority were represented by *M. scutellatus* (Table 3).

A total of 5619 insects were examined for the presence of the pinewood nematode and only one specimen of *Monochamus clamator* collected in British Columbia contained the pinewood nematode ("m" form). The pinewood nematode did not occur in any other of the

1293 *Monochamus* specimens sampled; a genus known to be a frequent and potential vector of the pinewood nematode.

A total of 198 cerambycid beetles (other than *Monochamus*), 300 buprestid beetles, and 3391 scolytid beetles (bark beetles) sampled for pinewood nematode were negative (Table 3). Furthermore, no pinewood nematode was isolated from curculionids (weevils), siricids (wood wasps), or other insect species sampled (Table 3).

### Mill and Other Surveys

The hemlock logs and lumber sampled at 22 mills in New Brunswick and Nova Scotia (Fig. 7) were negative for the pinewood nematode. About 0.7% of the logs (n = 1766) contained cerambycid wood borer larvae, but these were not identified to genus. The pinewood nematode was absent in all 167 samples taken from the logs, including the samples with grub holes. Only six boards of the 188 lumber piles examined at two mill sites contained grub holes. These six and all other boards sampled (n = 28) did not contain the pinewood nematode.

Insect damage or activity was detected in 20% of the logs inspected in 1990 in British Columbia. However, samples examined in the laboratory contained neither *Monochamus* spp. nor pinewood nematode. All insects that emerged from the 96 log bolts artificially inoculated with high numbers of pinewood nematode were free of the pinewood nematode though nematodes of other genera were present in some of the insects.

About 80% of the 462 trees sampled near Goose Bay, Labrador were free of nematodes, but the pinewood nematode ("m" form) was isolated from five black spruce and four balsam fir (Fig. 8). Populations were extremely low and usually only one to three nematodes occurred in any of the nine positive samples.

### DISCUSSION

Results of this nationwide survey established that the pinewood nematode, *B. xylophilus*, is present in all provinces of Canada, except Prince Edward Island where it has not been detected. Although the pinewood nematode is transcontinental in Canada, it has not caused wilt disease and tree mortality. The incidence of the nematode ranged from low in Ontario to rare in British Columbia (Table 2). The fact that only nine healthy trees were positive for pinewood nematode with no pine wilt disease symptoms demonstrates that the Canadian situation does not compare with that of Japan. Our results corroborate earlier reports that the distribution of pinewood nematode cannot be equated with the occurrence of pine wilt disease (Wingfield *et al.* 1984; Dwinell and Nickle 1989).

The standardized methods used in this nationwide survey emphasized the sampling of both host and vector species most likely to harbour the pinewood nematode. Consequently, the results represent worst-case scenarios. The prioritized survey methods coupled with sample sizes of 3706 trees and 1294

*Monochamus* beetles (Tables 1-3), both having the greatest probability of containing the pinewood nematode, are considered adequate to establish the distribution and frequency of the nematode within potential host trees and insect vectors. Furthermore, the isolation of nematodes representing different genera from a large number of tree species suggests that the techniques and procedures used were reliable for the detection and isolation of the pinewood nematode.

The absence of pine wilt disease in Canada may be explained in part by the fact that the disease so far occurred predominantly in areas where the mean summer air temperature exceeds 20°C (Magnusson 1986; Rutherford and Webster 1987). Such a high threshold is expected to mitigate epidemic development of pine wilt disease in the cool climate of much of Europe, North America and Asia, even if the nematode is present (Rutherford and Webster 1987; Rutherford *et al.* 1990). Furthermore, it is noteworthy that in addition to the scattered distribution of *B. xylophilus* in Canadian forests, the number of nematodes in the wood samples and insect vectors were low, ranging from 0-10 (Finney-Crawley and Ebsary Pers. Comm.) Such low numbers are in sharp contrast to the several thousands reported by Linit (1988) and Humphry and Linit (1989).

Both the "m" and "r" forms of *B. xylophilus* are present in Canada, but the "m" form is more common and more widely distributed. The "r" form occurred only in New Brunswick, Nova Scotia, Quebec, Manitoba and Alberta (Table 2). The "r" form is almost exclusively limited to pines, whereas the "m" form is frequent

in fir and spruce. The "m" form conforms to the taxonomic description of the mucronated *B. xylophilus* described from Minnesota by Wingfield *et al.* (1983) and is considered less virulent than the "r" form. Inoculations of seedlings with isolates of "m" and "r" forms confirmed that although virulence is host dependent, the Canadian "m" form is less virulent than the "r" form (Futai and Sutherland 1989). Similarly in Europe, the mucronated species, *B. mucronatus*, has not been found pathogenic on unstressed pine (de Guiran and Bruguiere 1989).

The taxonomy of *B. xylophilus* and related species continues to undergo revision because of the high variability of form and pathogenicity associated with the species complex. Although the definition of species is not completed, three genetically controlled factors have traditionally been used to define nematode species: 1. the ability of species to hybridize with each other, 2. pathogenicity, and; 3. morphological features. De Guiran and Boulbria (1986) proposed a *supraspecies*, characterized by forms with incomplete reproductive isolation, to include all existing populations of pinewood nematode and hypothesized that the Japanese and American strains were derived from a common stock from western Europe. More recently, Webster *et al.* (1990) and Tares *et al.* (1992) have confirmed these affinities and relationships within the *supraspecies* using DNA probes to analyze genomic DNA hybridization patterns.

The pinewood nematode occurs in low numbers in six species of pine in Canada: Scots pine, jack pine, lodgepole pine, red pine, Ponderosa pine and eastern

white pine. The pinewood nematode also occurred in low numbers in six other common conifers: balsam fir, white spruce, black spruce, red spruce, tamarack and Douglas-fir. It was absent in hemlock and the various species of cedars. Within host trees, the nematode was usually present only in trees weakened by other factors. However, occasionally the nematode was present in healthy host trees. The immunity of western red cedar to *B. xylophilus* and its primary insect vector *Monochamus* spp. has been confirmed recently based on surveys and inoculation experiments (Singh *et al.* 1992).

The pinewood nematode was absent in all but one potential vector, including insects that had emerged from logs artificially inoculated with high numbers of nematodes. Successful isolation was made from only one specimen of *Monochamus clamator* collected from British Columbia which contained the "m" form of the nematode. Similarly, the pinewood nematode was absent from members of the Buprestidae and Curculionidae both of which have been implicated as potential vectors in the United States (Linit 1988). The failure to identify a vector for pinewood nematode, despite intensive sampling, implies that the frequency of transmission of the nematode to trees in Canada is probably low, and therefore the risk of accidental import by European countries small, especially with continued programs to eliminate bark and grub holes. Furthermore, the high threshold of 20°C mean daily temperatures required for disease expression is expected to mitigate epidemic development of pine wilt disease in much of Europe, even if the pinewood nematode is inadvertently introduced (Rutherford and Webster 1987; Webster

*et al.* 1990). Also noteworthy is that mucronated forms of the *Bursaphelenchus* have been documented in France (de Guiran and Boulbria 1986), Norway (McNamara and Stoen 1988), Finland (Tomminen *et al.* 1989) Sweden (Magnusson and Schroeder 1989) and Germany (Schauer-Blume and Sturhan 1989).

## CONCLUSIONS

The Forest Insect and Disease Survey of Forestry Canada conducted a nationwide survey to determine the distribution of the pinewood nematode in Canada. The survey included both tree and insect species that were considered potential host or vectors for the nematode. Results of the survey support several conclusions:

1. The survey methods prioritized and emphasized the sampling of both host and vector species most likely to harbour the pinewood nematode. Therefore, the results represent worst-case scenarios.
2. Sample sizes of 3706 trees, including 2773 dead and dying trees, and 1294 *Monochamus* beetles both having the greatest probability of containing the pinewood nematode, are considered adequate to establish the frequency of pinewood nematode within potential host trees and insect vectors.
3. The pinewood nematode is present in small isolated groups of one or two trees in the provinces of Canada with the exception of Prince Edward



- Island, where it has not been detected. The frequency of occurrence varied from being relatively low in Ontario to very low in Quebec and New Brunswick to rare in Newfoundland, Nova Scotia and Prairie Provinces to extremely rare in British Columbia.
4. Both the "r" and "m" forms of the pinewood nematode are present in Canada, but the "m" form was more common. The "r" form occurred only in New Brunswick, Nova Scotia, Quebec, Ontario, Manitoba and Alberta. The "r" form is almost exclusively limited to pine, whereas the "m" form is more likely to occur in fir and spruce.
  5. The pinewood nematode was present in low numbers in six species of pine in Canada: Scots pine, jack pine, lodgepole pine, red pine, Ponderosa pine and eastern white pine. The pinewood nematode also occurred in low numbers in six other common conifers: balsam fir, white spruce, black spruce, red spruce, tamarack and Douglas-fir. It is absent in hemlock and the various species of cedars. When present, the nematode most often occurred in trees weakened by other factors.
  6. A total of 5619 insects, including 1294 *Monochamus* sp., were examined for the pinewood nematode. The nematode was absent in all but one potential vector, including insects that had emerged from logs artificially inoculated with high numbers of nematodes. Successful isolation was made from only one specimen of *Monochamus clamator* collected from the field.
  7. The risk of importing the pinewood nematode with Canadian lumber and logs, or indirectly through vectors, is considered extremely low especially with continued programs to eliminate bark and grub holes. Furthermore, the high threshold temperatures (20°C+) required for the development of pine wilt disease would preclude disease expression in much of Europe. Moreover, mucronated forms of *Bursaphelenchus* are known to occur in France, Norway, Finland, Sweden and Germany.

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Table 1. Number of trees sampled for pinewood nematode (PWN) surveys in Canada from 1985 to 1990 by species and health status.

Province & Tree species	Health Status			Total
	Healthy	Moribund	Dead	
<b>Newfoundland (Island)</b>				524
Balsam fir	98	85	58	241
Black spruce	66	36	28	130
White spruce	31	6	6	43
Sitka spruce	8	6	3	17
Tamarack	55	3	7	65
Eastern white pine	7	7	1	15
Scots pine	4	4	4	12
Red pine	1			1
<b>Newfoundland (Labrador)</b>				154
Balsam fir	24	15	10	49
Black spruce	25	22	25	72
White spruce	3		1	4
Tamarack	15	7	7	29
<b>Nova Scotia</b>				135
Balsam fir	15		43	58
Red Spruce	4		4	8
White Spruce			6	6
Black spruce		20	9	29
Eastern hemlock	1		1	2
Red pine	5		1	6
Eastern white pine	13	3	5	21
Scots pine	1	2	2	5

Table 1 (cont'd.)

Province & Tree species	Health Status			Total
	Healthy	Moribund	Dead	
<b>Prince Edward Island</b>				55
Balsam fir	4		9	13
Black spruce	1		2	3
White spruce	9		21	30
Sitka spruce	1			1
Tamarack			1	1
Red pine	1			1
Scots pine	1	1		2
Eastern white pine	1	3		4
<b>New Brunswick</b>				238
Balsam fir	20		88	108
Black spruce	5		17	22
White spruce	3		11	14
Red spruce	2		6	8
Red pine	1		5	6
Jack pine	6		41	47
Scots pine	3		8	11
Eastern white pine			17	17
Eastern hemlock			4	4
Eastern red cedar			1	1
<b>Quebec</b>				340
Balsam fir	10	6	89	105
Red spruce			1	1
White spruce	1	1		2
Black spruce			2	2
Tamarack	2	1		3
Eastern white pine	1	16	43	60
Jack pine	1	15	47	63
Red pine	6	24	30	60
Scots pine		24	19	43
Eastern white cedar			1	1

Table 1 (cont'd.)

Province & Tree species	Health Status			Total
	Healthy	Moribund	Dead	
<b>Ontario</b>				<b>642</b>
Balsam fir		5	114	119
Black spruce		5	52	57
White spruce		4	23	27
Norway spruce			5	5
Tamarack			2	2
Eastern white pine	3	14	32	49
Scots pine	2	1	13	16
Jack pine	2	34	213	249
Red pine	6	38	71	115
Austrian pine		1	2	3
<b>Prairie Provinces</b>				<b>320</b>
Samples were tallied by host and segregated into two categories: Healthy and Dead (all recently dead or dying), with equal samples of each. Hosts included: balsam fir, black spruce, white spruce, lodgepole pine, jack pine, red pine, eastern white pine, limber pine, Douglas-fir, and tamarack.	160		160	320

Table 1 (concl'd.)

Province & Tree Species	Health Status			Total
	Healthy	Moribund	Dead	
<b>British Columbia</b>				<b>1298</b>
Amabilis fir	8	4	49	61
Alpine fir	28	28	21	77
Grand fir		1	1	2
White spruce	7	13	51	71
Black spruce	1	1		2
Engelmann spruce		1	3	4
Sitka spruce	2	1		3
Tamarack	1	1		2
Western larch	1	2	2	5
Western hemlock	43	41	108	192
Douglas-fir	25	21	77	123
Western white pine	14	7	10	31
Lodgepole pine	124	166	198	488
Scots pine	2	1	1	4
Ponderosa pine	9	5	5	19
Whitebark pine	1	1	1	3
Western red cedar	36	36	105	177
Yellow cypress	3	8	15	26
Trembling aspen		1		1
Mixed species			7	7
<b>Grand Total</b>	<b>933</b>	<b>748</b>	<b>2025</b>	<b>3706</b>

Table 2. Incidence of the pinewood nematode (PWN) in Canada from 1985 to 1990 by species and health status of the host.

Tree Species	Form of PWN			Host Status*			Number of		No. Locations with PWN
	"m" form	"r" form	undetermined	H	M	D	Trees with PWN**	Trees Sampled	
<b>Newfoundland (Island)</b>							14	436	8
Balsam fir	7				2	5	7	241	
Black spruce	2			1		1	2	130	
Tamarack	5			5			5	65	
<b>Newfoundland (Labrador)***</b>								154	
All hosts									
<b>Nova Scotia****</b>							11	113	9
Balsam fir	7					7	7	58	
Black spruce	2					2	2	29	
Eastern white pine	1					1	1	21	
Scots pine	1					1	1	5	
<b>Prince Edward Island</b>								55	
All hosts									
<b>New Brunswick</b>							21	180	18
Balsam fir	12					12	12	108	
Red spruce	1					1	1	8	
Jack pine	1	6				7	7	47	
Eastern white pine		1				1	1	17	
<b>Quebec</b>							48	331	47
Balsam fir	23			1	2	20	23	105	
Eastern white pine	2	2		1		3	4	60	
Red pine	2				1	1	2	60	
Scots pine	2	3				5	5	43	
Jack pine	5	9		1		13	14	63	

Table 2 (concl'd.)

Tree Species	Form of PWN			Host Status*			Number of		No. Locations with PWN
	"m" form	"r" form	undetermined	H	M	D	Trees with PWN**	Trees Sampled	
<b>Ontario</b>							221	632	180
Balsam fir	21	3	12		2	33	35	119	
Black spruce	12		4		1	15	16	57	
White spruce	3	2			1	2	3	27	
Jack pine	11	37	77		12	105	117	249	
Red pine	3	12	18		13	20	33	115	
Scots pine		3	1		1	3	4	16	
Eastern white pine	1	9	3		3	10	13	49	
<b>Manitoba</b>							3		3
Jack pine		3			3		3		
<b>Saskatchewan</b>							2		2
White spruce	2				2		2		
<b>Alberta</b>							4		4
Balsam fir	2					2	2		
Jack pine		1				1	1		
Lodgepole pine		1				1	1		
<b>British Columbia</b>							6	701	6
Lodgepole pine	3				1	2	3	488	
Ponderosa pine			1			1	1	19	
Douglas-fir	1					1	1	123	
White spruce			1			1	1	71	
<b>Grand Total</b>	132	92	117	9	44	277	330		277

\* H = Healthy, M = Moribund, D = Dead

\*\* Number in rows may exceed the total because at times the "r" and "m" forms occurred in the same sample.

\*\*\* The pinewood nematode was not isolated in Labrador in this tree survey, but sampling in 1991 detected the "m" form.

\*\*\*\* The "r" form was not isolated in Nova Scotia in this tree survey, but inspection of lumber samples showed the presence of this form.

Table 3. Number of potential insect vectors sampled for pinewood nematode (PWN) in Canada by province from 1987 to 1990.

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<b>Cerambycidae</b>											
<i>Monochamus marmorator</i>					2						2
<i>Monochamus scutellatus</i>	18	19	1	243	101	6			17	308	713
<i>Monochamus clamator</i>										68	68
<i>Monochamus notatus</i>		2			1					8	11
<i>Monochamus titillator</i>				2							2
<i>Monochamus spp.</i>		3		6	253	236					498
<b>Total <i>Monochamus</i></b>	<b>18</b>	<b>24</b>	<b>1</b>	<b>251</b>	<b>357</b>	<b>242</b>			<b>17</b>	<b>384</b>	<b>1294</b>
<i>Arhopalus foveicollis</i>	52			1							53
<i>Arhopalus asperatus</i>										1	1
<i>Asemum striatum</i>				9						7	16
<i>Cosmosalia chrysocoma</i>				1						2	3
<i>Graphisurus sp.</i>				1							1
<i>Pygoleptura nigrella</i>				1						1	2
<i>Pygoleptura brevifrons</i>		1									1
<i>Pygoleptura spp.</i>				3							3
<i>Acmaeops proteus</i>				1						7	8
<i>Cortodera longicornis</i>										2	2

Table 3 (cont'd.)

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<i>Lepturopsis dolorosa</i>										1	1
<i>Judolia montivagus</i>										1	1
<i>Neoclytus muricatus</i>										4	4
<i>Tetropium velutinum</i>										1	1
<i>Semanotus ligneus</i>										16	16
<i>Bellamira scularis</i>				1							1
<i>Neacanthocinus obliquus</i>										13	13
<i>Pachyta lamed</i>										5	5
<i>Strictoleptura canadensis</i>		1		3						14	18
<i>Rhagium inquisitor</i>					1					4	5
<i>Xestoleptura crassipes</i>										2	2
<i>Xylotrechus undulatus</i>	1	3			6				3	14	27
Cerambycidae (undet.)	1				6				7		14
<b>Total Cerambycidae (other than <i>Monoctonus</i>)</b>	<b>54</b>	<b>5</b>		<b>21</b>	<b>13</b>				<b>10</b>	<b>95</b>	<b>198</b>



Table 3 (cont'd.)

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<b>Buprestidae</b>											
<i>Buprestis lyrata</i>						2				60	62
<i>Buprestis nuttali</i>										29	29
<i>Buprestis langi</i>										2	2
<i>Buprestis rusticorum</i>										6	6
<i>Buprestis maculativentris</i>				6							6
<i>Chalcophora virginiensis</i>										3	3
<i>Chrysobothris dentipes</i>				2							2
<i>Chrysobothris trinervia</i>										2	2
<i>Chrysobothris scabripennis</i>				4							4
<i>Chrysobothris spp.</i>		8	1	3						4	16
<i>Dicerca tenebrosa</i>		2		2						28	32
<i>Dicerca spp.</i>		21		2							23
<i>Melanophila spp.</i>		2	3	1						4	10
<i>Poecilonota cyanipes</i>		1									1
Buprestidae (undet.)	41	9			34	13			5		102
<b>Total Buprestidae</b>	41	43	4	20	34	15			5	138	300

Table 3 (cont'd.)

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<b>Scolytidae</b>											
<i>Dendroctonus ponderosae</i>										25	25
<i>Dendroctonus simplex</i>			9	63							72
<i>Dendroctonus murrayanae</i>									1		1
<i>Dendroctonus rufipennis</i>		120							15		135
<i>Dendroctonus valens</i>									4		4
<i>Dryocoetes confusus</i>										8	8
<i>Dryocoetes affaber</i>				3							3
<i>Hylurgops rufipennis</i>									10	19	29
<i>Hylurgops porosus</i>										17	17
<i>Ips pini</i>						9					9
<i>Ips borealis</i>		566	8	20							594
<i>Ips spp.</i>	1				128				41		170
<i>Orthotomicus caelatus</i>		15		3							18
<i>Polygraphus rufipennis</i>	27	170		22					5		224
<i>Pityokteines sparsus</i>		11		87							98
<i>Trypodendron lineatum</i>		1		11							12
Scolytidae (undet.)	33	82	12		682	1157			6		1972
<b>Total Scolytidae</b>	<b>61</b>	<b>965</b>	<b>29</b>	<b>209</b>	<b>810</b>	<b>1166</b>			<b>82</b>	<b>69</b>	<b>3391</b>

Table 3 (cont'd.)

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<b>Curculionidae</b>											
<i>Cylindrocopterus spp.</i>										12	12
<i>Hylobius warreni</i>	2										2
<i>Hylobius congener</i>		7		2							9
<i>Hylobius spp.</i>	7					18					25
<i>Pissodes dubius</i>				8							8
<i>Pissodes spp.</i>	2				10					7	19
Curculionidae (undet.)	12	4									16
<b>Total Curculionidae</b>	<b>23</b>	<b>11</b>		<b>10</b>	<b>10</b>	<b>18</b>				<b>19</b>	<b>91</b>
<b>Melandryidae</b>											
<i>Serropalpus barbatus</i>				2							2
<b>Ostomidae</b>											
<i>Temnochila chlorodia</i>										2	2
<b>Cleridae</b>											
<i>Thanasimus undatulus</i>	3								4		7

Table 3 (concl'd.)

Potential Vector	Province and Number of Insects Sampled										
	NF	NS	PEI	NB	PQ	ON	MB	SK	AB	BC	Total
<b>Elateridae</b>											
<i>Ctenicera spp.</i>	2										2
Elateridae (undet.)	34					3					37
Misc. <i>Coleoptera</i>	55									91	146
<b>Siricidae</b>											
<i>Sirex cyaneus</i>										4	4
<i>Sirex juvencus</i>										1	1
<i>Sirex spp.</i>	3	4									7
<i>Urocerus albicornis</i>		5		6	36					5	52
<i>Urocerus gigas</i>										6	6
<i>Urocerus spp.</i>					8					1	9
<i>Xeris spectrum</i>										3	3
Siricidae (undet.)									1		1
<b>Total Siricidae</b>	3	9		6	44				1	20	83
<b>Formicidae</b>											
<i>Camponotus herculeanus</i>	9								2		11
Miscellaneous	44			1		10					55
<b>Grand Total</b>	347	1057	34	520	1268	1454			121	818	5619

Table 4. Nematode genera isolated in Canada from 1985 to 1990 by host tree species, health status and province.<sup>1</sup>

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Anaplectus</i>														
Black spruce			+								+			
<i>Anquillonema</i>														
Jack pine			+									+		
<i>Aphelenchoides</i>														
Balsam fir	+	+	+	+	+	+		+	+	+				
Black spruce	+	+	+	+	+			+	+	+	+	+		
Red spruce		+						+						
White spruce	+	+	+	+				+		+		+		
Tamarack	+	+	+	+		+		+	+					
Jack pine			+							+				
Red pine			+							+				
Eastern white pine			+							+				
<i>Aphelenchus</i>														
Balsam fir		+		+										
Black spruce			+						+					

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Bursaphelenchus xylophilus</i>														
Balsam fir	+	+	+	+		+		+	+	+			+	
White spruce		+	+							+		+		+
Black spruce	+	+	+	+		+				+				
Red spruce			+					+						
Tamarack	+		+	+										
Douglas-fir			+											+
Eastern white pine	+	+	+			+		+	+	+				
Jack pine	+	+	+					+	+	+	+		+	
Scots pine		+	+			+			+	+				
Lodgepole pine		+	+										+	+
Red pine		+	+						+	+				
Ponderosa pine			+											+
<i>Bursaphelenchus</i> (not pinewood nematode)														
Black spruce		+	+	+		+				+				
Eastern white spruce			+							+				
Eastern white pine			+			+				+				
Jack pine			+					+		+				
Red pine	+		+					+		+				
Lodgepole pine		+	+											+
Western white pine	+													+

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Caenrhabdites</i>														
Balsam fir			+					+						
<i>Cryptaphelenchoides</i>														
Balsam fir			+	+						+				
Scots pine		+	+	+										
<i>Cryptaphelenchus</i>														
Balsam fir	+		+	+				+	+					
White spruce	+			+										
Black spruce	+		+	+								+	+	
Eastern white pine		+	+	+		+								
Jack pine			+							+				
Red pine		+								+				
<i>Deladenus</i>														
Balsam fir	+	+	+	+	+				+	+			+	
Black spruce	+	+	+	+	+				+				+	
Tamarack	+			+										
Red pine	+									+				
Eastern white pine			+			+			+					
<i>Diplogaster</i>														
Balsam fir		+	+	+		+								

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Diploscapter</i>														
Balsam fir			+						+					
<i>Ditylenchus</i>														
Jack pine			+							+				
Eastern white pine			+			+								
<i>Dotylophus</i>														
Balsam fir		+	+					+	+					
Black spruce			+										+	
Red pine		+	+						+	+				
<i>Ektaphelenchus</i>														
Balsam fir		+	+	+	+	+		+	+				+	
Black spruce		+		+										
White spruce			+									+		
Tamarack	+	+		+										
Eastern white pine	+		+	+						+				
<i>Heterocephalobus</i>														
Balsam fir			+										+	
<i>Hexatyloid</i>														
Balsam fir			+					+						
Eastern white pine			+					+						



Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Koernaria</i>														
White spruce	+			+										
Eastern larch	+			+										
<i>Laimaphelenchus</i>														
Balsam fir	+		+	+					+					
White spruce			+									+	+	
Black spruce	+		+	+								+	+	
Tamarack	+			+					+					
Jack pine			+								+	+	+	
<i>Macrolaimas</i>														
Balsam fir			+						+	+			+	
Black spruce			+									+		
White spruce			+									+		
Tamarack	+								+					
Jack pine			+									+	+	
<i>Mesorhabdites</i>														
Balsam fir			+						+					
Mixed species			+											+

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Mikolletzka</i>														
Eastern white pine			+							+				
<i>Mononchoides</i>														
Red pine			+						+					
Eastern white pine			+							+				
<i>Neodiplogaster</i>														
Red pine			+							+				
Mixed species			+											+
<i>Neoditylenchus</i>														
Balsam fir			+					+	+	+				
Black spruce			+					+				+	+	
<i>Neotylenchus</i>														
White spruce			+									+		
<i>Nexatylus</i>														
Balsam fir		+	+						+					
<i>Nothotylenchus</i>														
Balsam fir			+					+						
<i>Panagrellus</i>														
Balsam fir			+						+					
<i>Panagrobellus</i>														
Balsam fir	+		+					+	+					

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Panagrolaimus</i>														
Balsam fir	+		+					+	+					
Jack pine			+									+		
<i>Panagromacra</i>														
Balsam fir			+									+		
<i>Parasitaphelenchus</i>														
Balsam fir			+										+	
Jack pine			+							+				
Eastern white pine			+							+				
<i>Parasitorhabdites</i>														
Balsam fir			+	+					+				+	
Black spruce			+	+										
White spruce		+		+										
Red pine		+								+				
<i>Parasitylenchus</i>														
Balsam fir			+						+	+				
Red pine		+	+						+	+				
<i>Plectonchus</i>														
Eastern white pine			+							+				

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Paurodontella</i>														
Balsam fir	+								+					
<i>Paurodontus</i>														
Balsam fir			+						+					
<i>Peloacra</i>														
Balsam fir			+						+					
<i>Plectus</i>														
Balsam fir			+	+		+		+					+	
Black spruce	+		+	+										
<i>Poikilolaimus</i>														
Mixed species			+											+
<i>Prothallonema</i>														
Black spruce			+			+								
Jack pine			+					+						
<i>Protorhabdites</i>														
Balsam fir			+	+									+	
Black spruce			+								+			
<i>Pseudhalenchus</i>														
Balsam fir			+						+					

Table 4 (cont'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Rhabdites</i>														
Balsam fir			+									+		
Black spruce		+	+	+	+									
White spruce			+	+										
Tamarack	+		+	+										
Eastern white pine			+	+										
Scots pine			+	+										
<i>Rhabdontolaimus</i>														
Balsam fir	+	+	+			+		+	+	+			+	
Black spruce			+							+				
White spruce			+							+		+		
Jack pine			+							+				
Red pine	+	+	+							+				
Eastern white pine			+					+		+				
Scots pine			+			+				+				
<i>Robleus</i>														
Red spruce			+			+								
<i>Seinura</i>														
Jack pine			+							+				
Red pine			+							+				
Eastern white pine			+							+				

.Table 4 (concl'd.)

Nematode genus and host tree species	Host Tree Status			Province										
				Newfoundland										
	Healthy	Morib.	Dead	Island	Lab	NS	PEI	NB	PQ	ON	MB	SK	AB	BC
<i>Sphaerularia</i>														
Balsam fir			+						+					
Jack pine			+									+		
<i>Teratocephalus</i>														
Tamarack			+		+									
<i>Tylaphelenchus</i>														
White spruce			+	+										
Tamarack	+			+										
Eastern white pine	+		+	+										
<i>Tylenchid</i>														
Balsam fir			+					+						
Eastern white pine			+			+								

<sup>1</sup> British Columbia samples collected from 1980 to 1990.

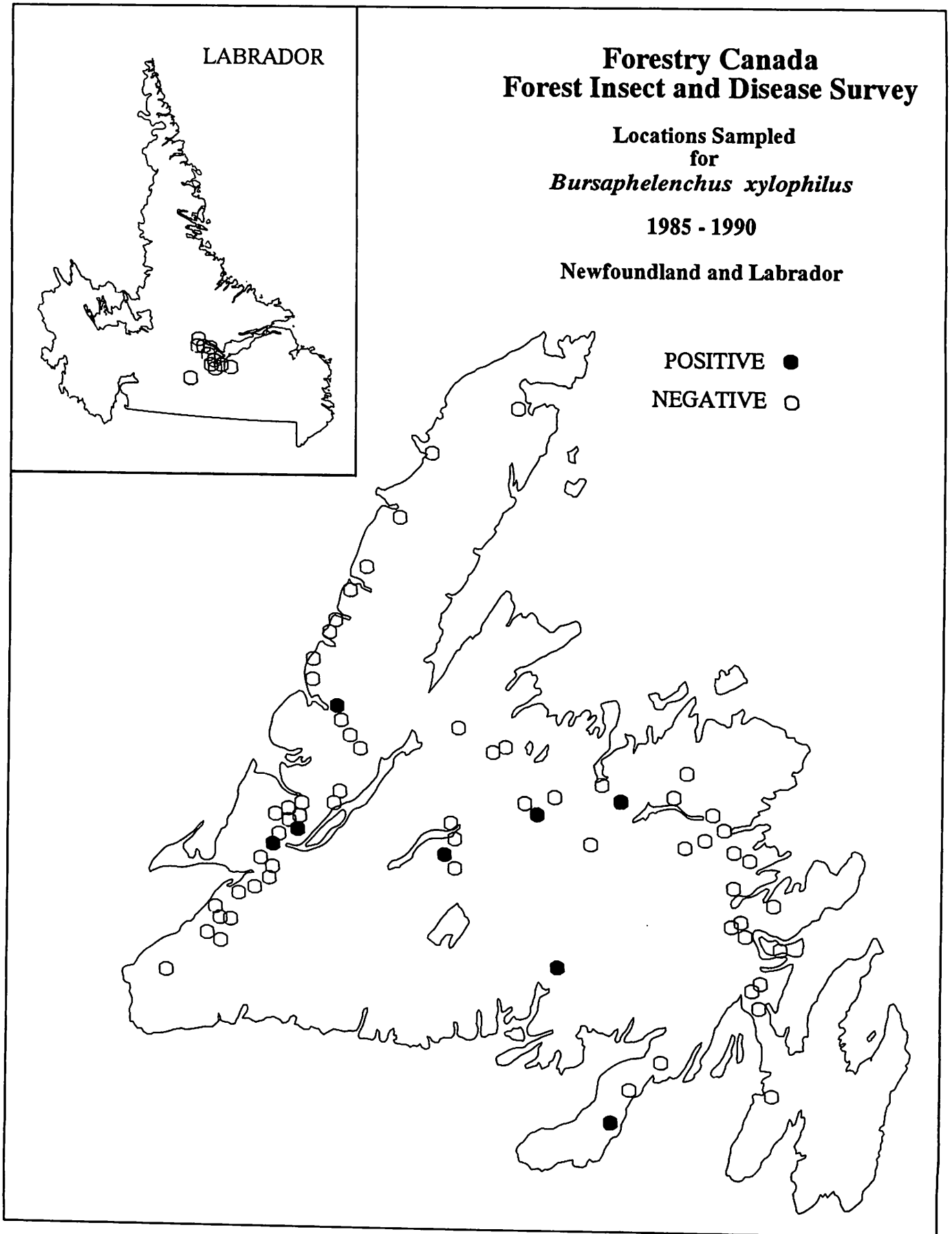


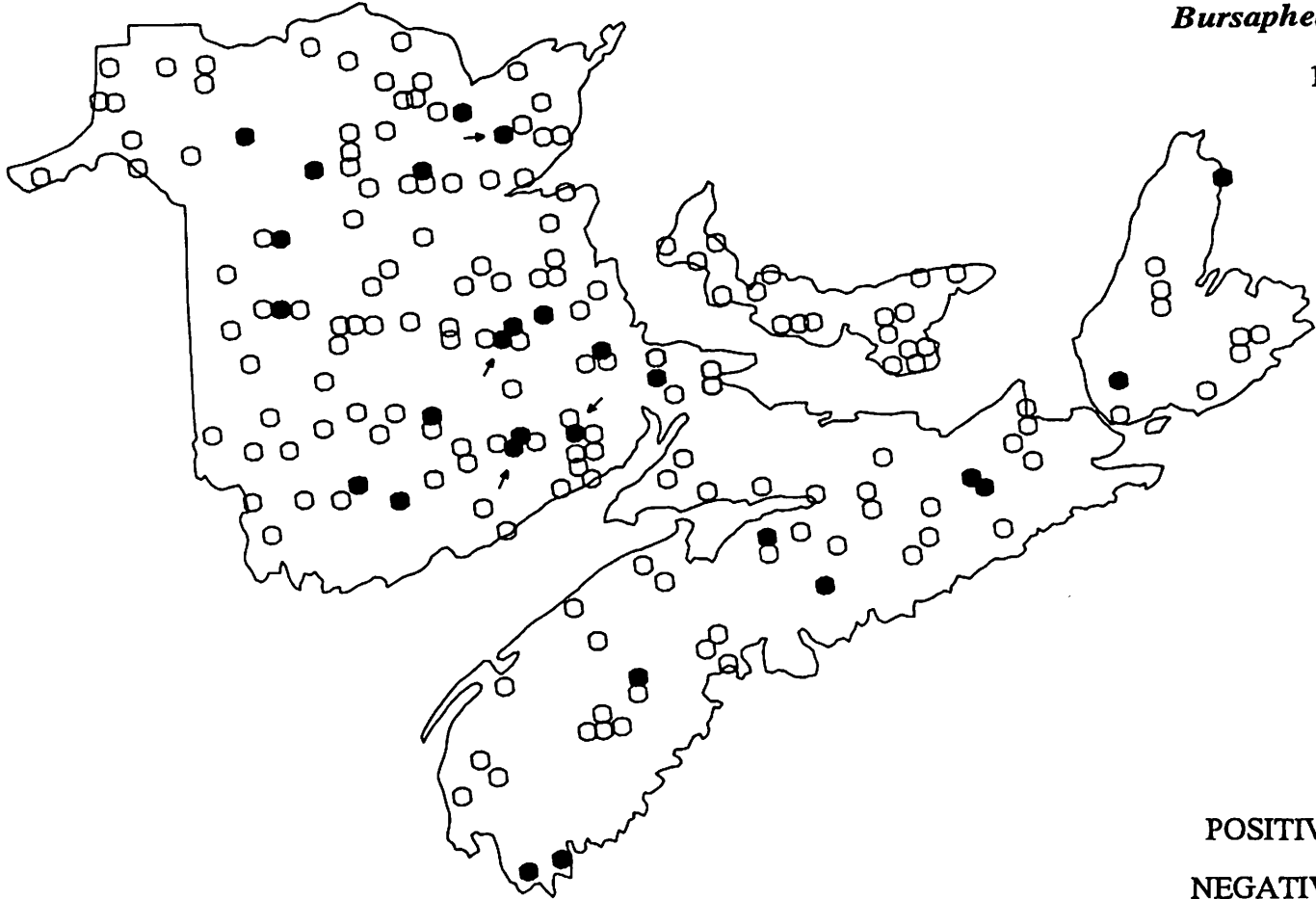
Figure 1. Tree sampling locations and isolations of pinewood nematode in Newfoundland from 1985 to 1990.

**Forestry Canada  
Forest Insect and Disease Survey**

**Locations Sampled  
for  
*Bursaphelenchus xylophilus***

**1985 - 1990**

**Maritimes**



**POSITIVE ● (→ = r form)**

**NEGATIVE ○**

Figure 2. Tree sampling locations and isolations of pinewood nematode in the Maritime Provinces from 1985 to 1990.



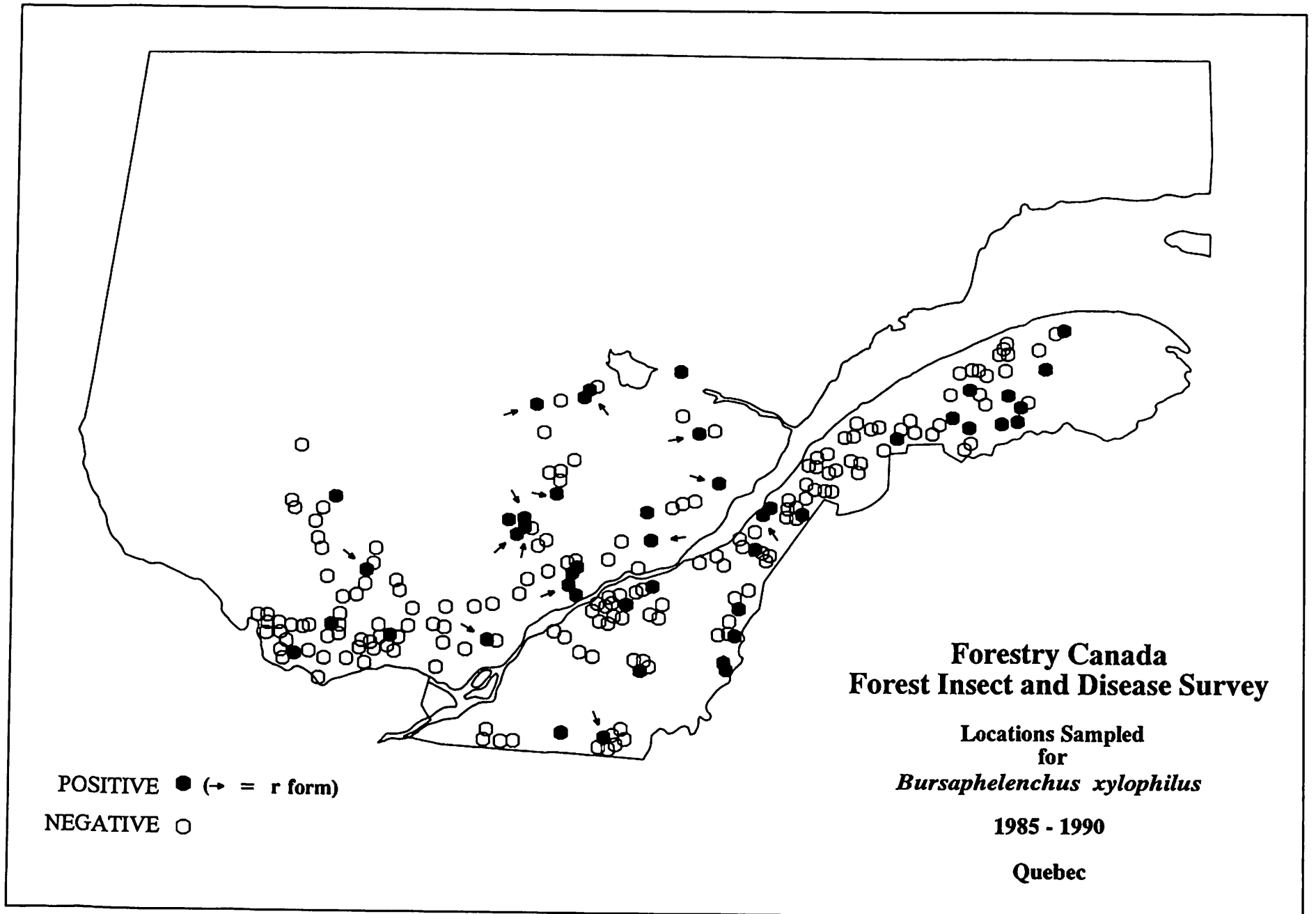


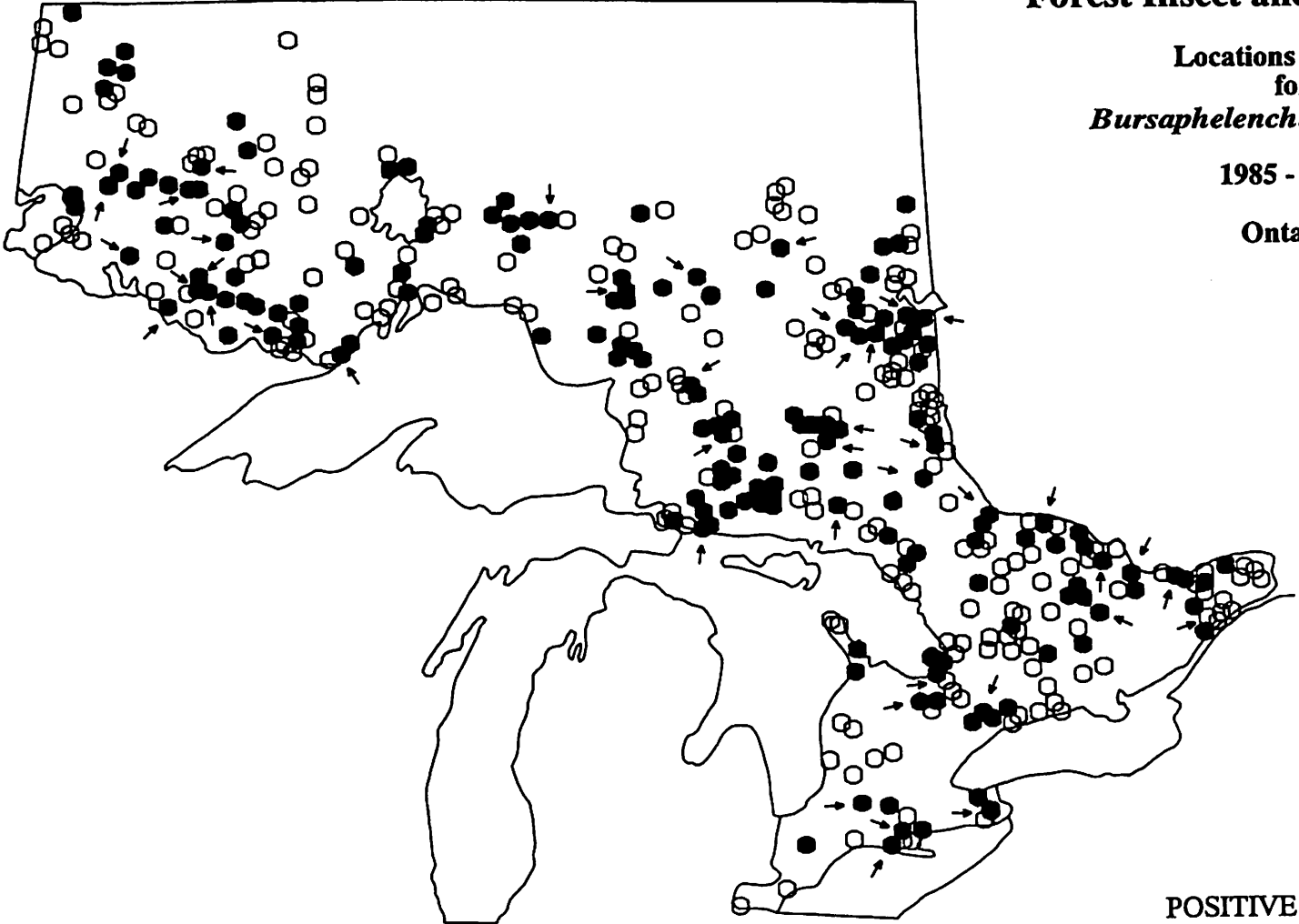
Figure 3. Tree sampling locations and isolations of pinewood nematode in Quebec from 1985 to 1990.

Forestry Canada  
Forest Insect and Disease Survey

Locations Sampled  
for  
*Bursaphelenchus xylophilus*

1985 - 1990

Ontario



POSITIVE ● (→ = r form)  
NEGATIVE ○

Figure 4. Tree sampling locations and isolations of pinewood nematode in Ontario from 1985 to 1990.

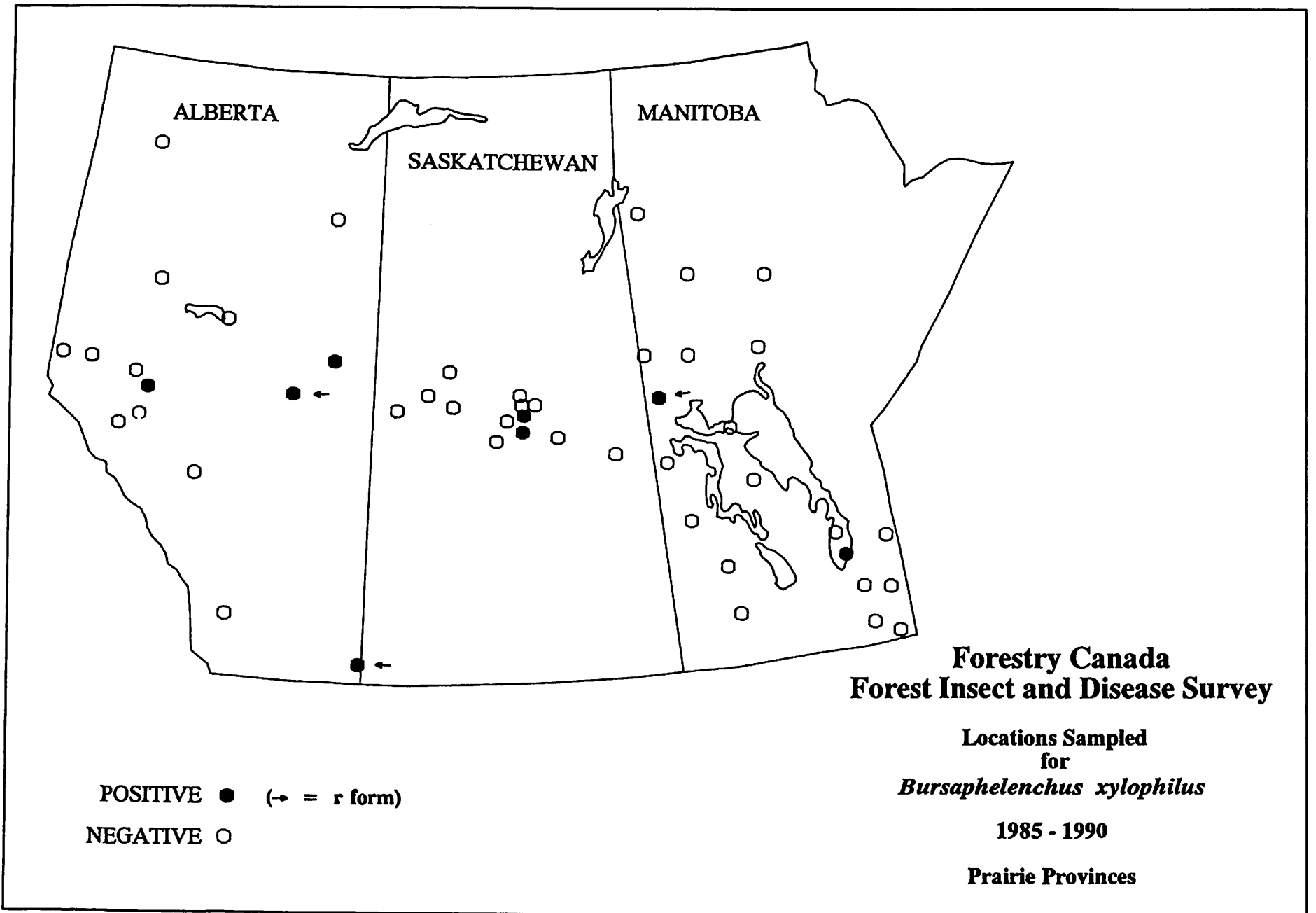


Figure 5. Tree sampling locations and isolations of pinewood nematode in the Prairie Provinces from 1985 to 1990.

**Forestry Canada  
Forest Insect and Disease Survey**

**Locations Sampled  
for  
*Bursaphelenchus xylophilus***

**1985 - 1990**

**British Columbia and Yukon**

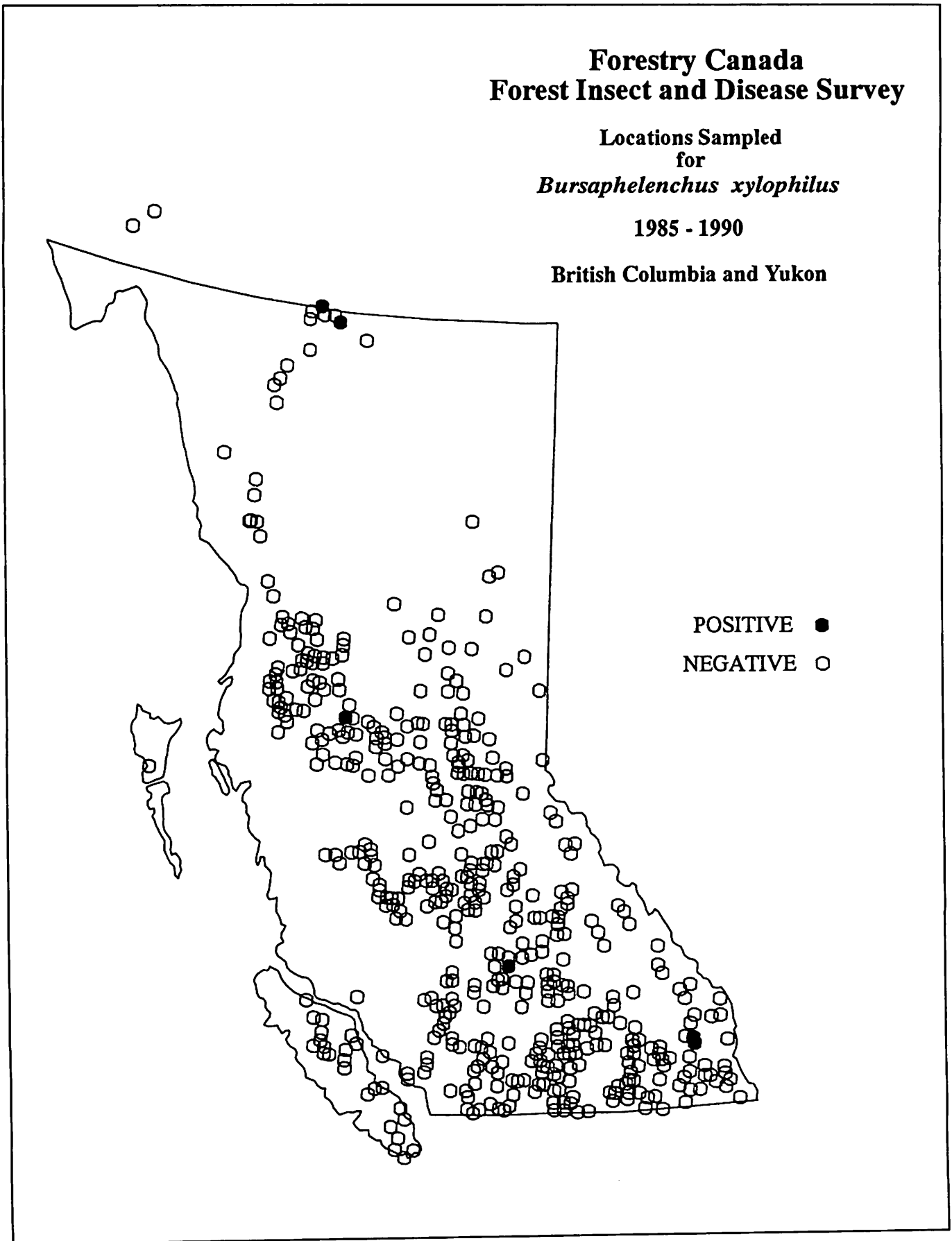


Figure 6. Tree sampling locations and isolations of pinewood nematode in British Columbia and Yukon from 1985 to 1990.

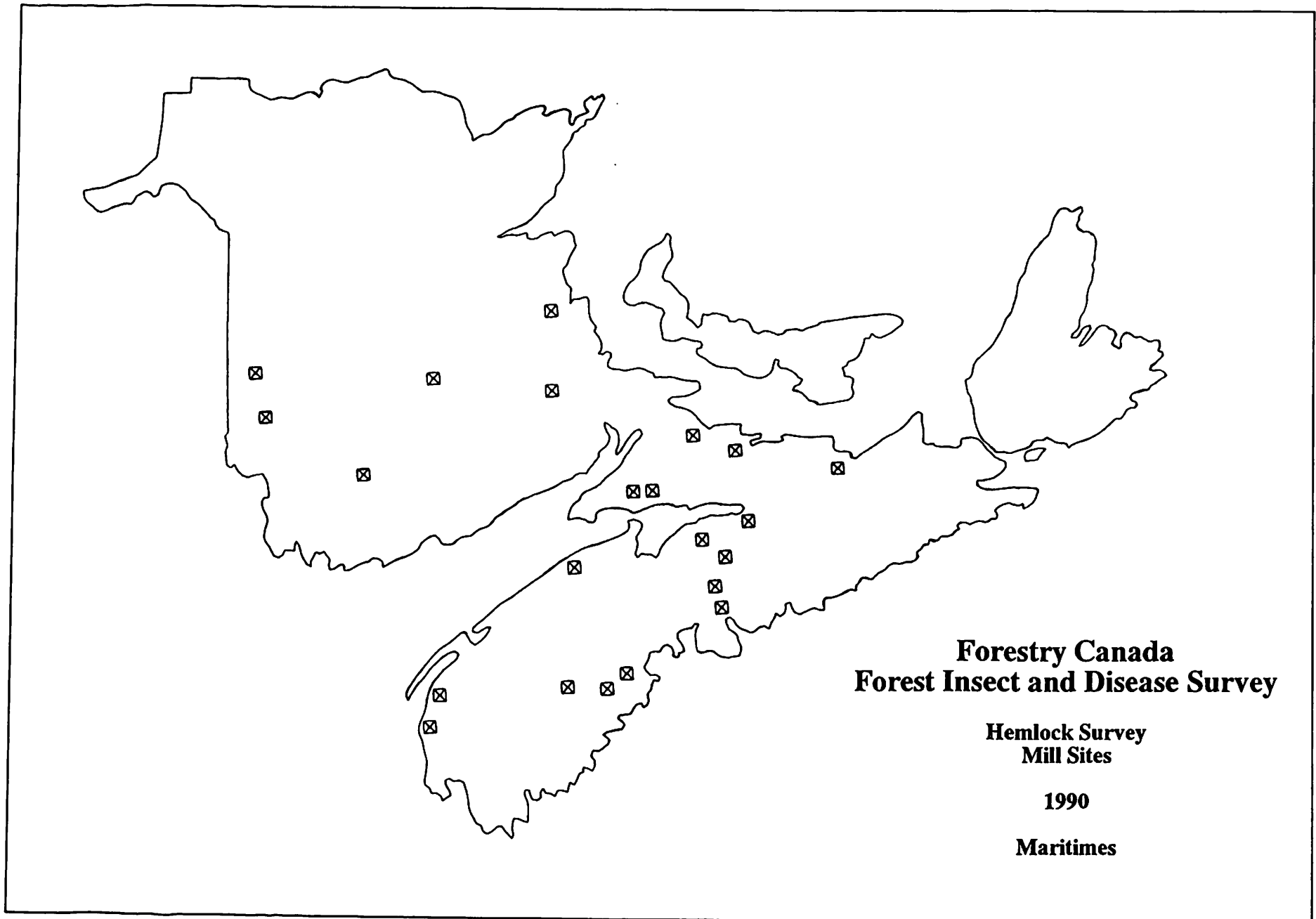


Figure 7. Locations of mills in the Maritime Provinces with hemlock logs or hemlock lumber sampled for pinewood nematode in 1990.

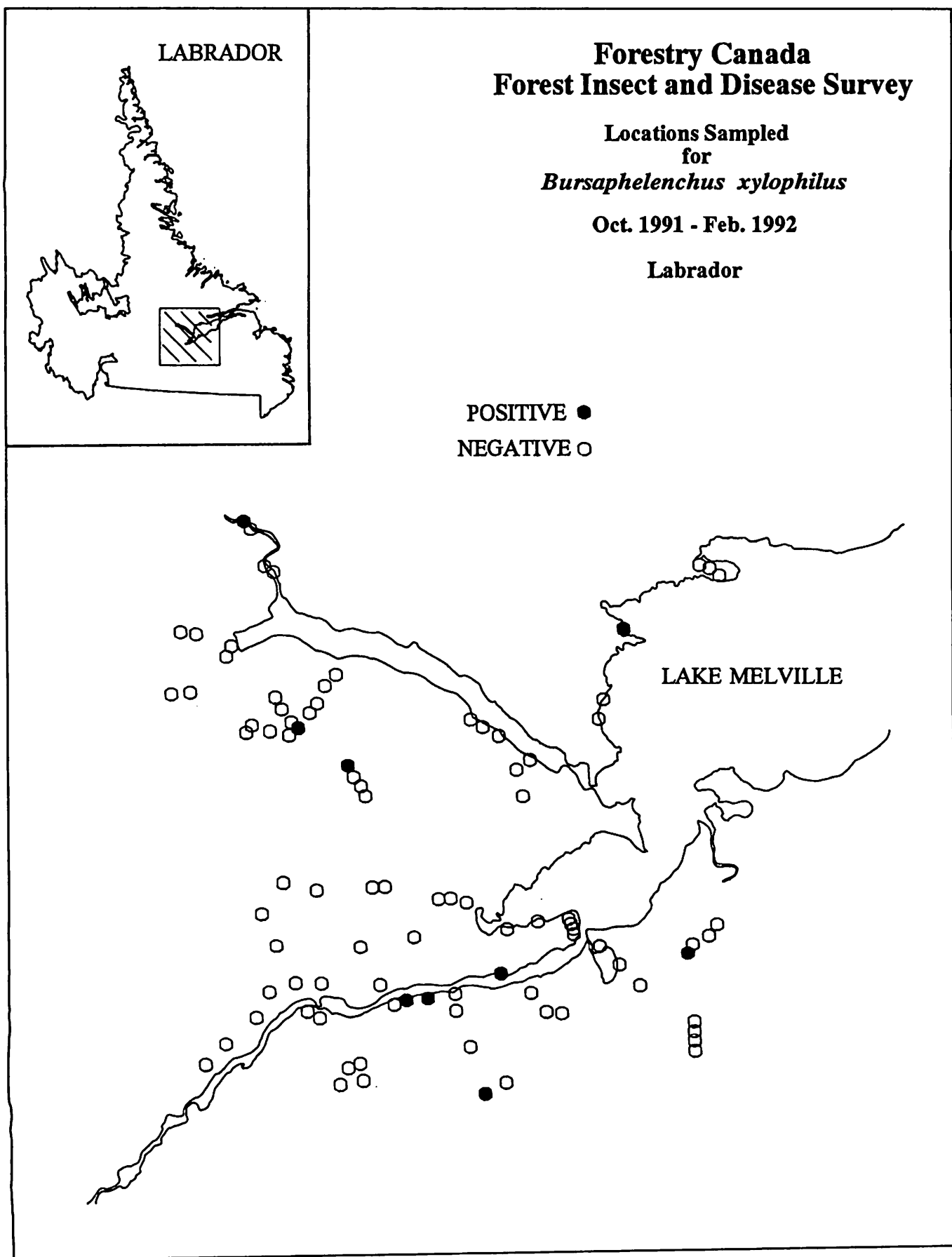


Figure 8. Tree sampling locations and isolations of pinewood nematode in Labrador from October 1991 to February 1992.

APPENDIX 1

COMMON AND SCIENTIFIC NAMES OF TREE SPECIES IN THIS REPORT

Balsam fir	<i>Abies balsamea</i> (L.) Mill.
Alpine fir	<i>Abies lasiocarpa</i> (Hook.) Nutt.
Amabilis fir	<i>Abies amabilis</i> (Dougl.) Forbes
Grand fir	<i>Abies grandis</i> (Dougl.) Lindl.
White spruce	<i>Picea glauca</i> (Moench) Voss
Black spruce	<i>Picea mariana</i> (Mill.) B.S.P.
Red Spruce	<i>Picea rubens</i> Sarg.
Engelmann Spruce	<i>Picea engelmannii</i> Parry
Sitka Spruce	<i>Picea sitchensis</i> (Bong.) Carr.
Norway spruce	<i>Picea abies</i> (L.) Karst.
Douglas-fir	<i>Pseudotsuga menziesii</i> (Mirbel) Franco
Armand pine	<i>Pinus armandii</i> Franch.
Eastern white pine	<i>Pinus strobus</i> L.
Western white pine	<i>Pinus monticola</i> Dougl.
Whitebark pine	<i>Pinus albicaulis</i> Engelm.
Limber pine	<i>Pinus flexilis</i> James
Jack pine	<i>Pinus banksiana</i> Lamb.
Japanese red pine	<i>Pinus densiflora</i> Sieb. and Zucc.
Japanese black pine	<i>Pinus thunbergii</i> Parl.
Lodgepole pine	<i>Pinus contorta</i> Dougl.
Red pine	<i>Pinus resinosa</i> Ait.
Ponderosa pine	<i>Pinus ponderosa</i> Laws.
Scots pine	<i>Pinus sylvestris</i> L.
Slash pine	<i>Pinus elliotii</i> Engelm.
Austrian pine	<i>Pinus nigra</i> Arnold
Tamarack (eastern larch)	<i>Larix laricina</i> (Du Roi) K. Koch
Western larch	<i>Larix occidentalis</i> Nutt.
Eastern hemlock	<i>Tsuga canadensis</i> (L.) Carr.
Western hemlock	<i>Tsuga heterophylla</i> (Raf.) Sarg.
Eastern red cedar	<i>Juniperus virginiana</i> L.
Eastern white cedar	<i>Thuja occidentalis</i> L.
Western red cedar	<i>Thuja plicata</i> Donn
Yellow cypress	<i>Chamaecyparis nootkatensis</i> (D. Don) Spach
Trembling aspen	<i>Populus tremuloides</i> Michx.

APPENDIX 2

LIST OF POTENTIAL VECTORS OF THE PINEWOOD NEMATODE  
AND PRIORITIES FOR COLLECTION IN CANADA

A. List of Genera of Potential Vectors

- |                        |   |   |
|------------------------|---|---|
| 1) <b>Cerambycidae</b> | <i>Monochamus</i><br><i>Asemum</i><br><i>Arhopalus</i><br><i>Rhagium</i><br><i>Acanthocinus</i><br><i>Xylotrechus</i><br><i>Neoclytus</i> | <b>Scolytidae (cont.)</b><br><i>Gnathotrichus</i><br><i>Scolytus</i><br><i>Orthotomicus</i><br><i>Pityokteines</i><br><i>Polygraphus</i><br><i>Dryocetes</i><br><i>Hylastes</i><br><i>Hylurgops</i> |
| 2) <b>Buprestidae</b>  | <i>Melanophila</i><br><i>Chrysobothris</i><br><i>Chalcophora</i><br><i>Buprestis</i><br><i>Dicerca</i><br><i>Trachykele</i>               | 5) <b>Siricidae</b><br><i>Sirex</i><br><i>Urocerus</i><br><i>Xeris</i>  |
| 3) <b>Melandryidae</b> | <i>Serropalpus</i>  | 6) <b>Curculionidae</b><br><i>Hylobius</i><br><i>Pissodes</i>   |
| 4) <b>Scolytidae</b>   | <i>Dendroctonus</i><br><i>Ips</i><br><i>Trypodendron</i>  | 7) <b>Miscellaneous</b><br>Cleridae<br>Ostomidae<br>Colydiidae  |



**B. Priorities for Collection of Potential Vectors**

**Priority I: *Monochamus* spp.**

<i>Monochamus scutellatus</i> (= <i>oregonensis</i> )	All Regions
<i>Monochamus notatus</i>	Maritimes, Quebec, Ontario, Prairies
<i>Monochamus marmorator</i>	Quebec, Ontario, Prairies
<i>Monochamus mutator</i>	Quebec, Ontario
<i>Monochamus carolinensis</i>	Ontario (southern)
<i>Monochamus clamator</i>	British Columbia

**Priority II: Primary Bark Beetles and Wood borers.**

Cerambycids from pines, spruce and firs	All Regions
Siricids - wood wasps	All Regions
<i>Dendroctonus</i> spp.	All Regions
<i>Ips</i> spp.	All Regions
<i>Pityokteines</i> spp.	All Regions
<i>Scolytus ventralis</i>	British Columbia
<i>Dryocetes confusus</i>	British Columbia
<i>Trypodendron</i> sp.	All Regions
<i>Gnathotrichus</i> spp.	All Regions
<i>Hylastes</i> spp.	All Regions
<i>Hylurgops</i> spp.	All Regions

**Priority III: Secondary Bark Beetles and Wood borers.**

Cerambycids from other conifers	All Regions
Buprestids from all conifers	All Regions
<i>Serropalpus</i> spp.	All Regions
<i>Hylobius</i> spp.	All Regions
<i>Pissodes</i> spp.	All Regions
Scolytids from all conifers	All Regions

**Priority IV: Other Bark-inhabiting Insects**

Clerids (adults only)	All Regions
Ostomids (adults only)	All Regions
Elaterids (adults only)	All Regions