THE ASSOCIATION AND TRANSMISSION OF *LEPTOGRAPHIUM PROCERUM* (KENDR.) WING., BY ROOT FEEDING INSECTS IN CHRISTMAS TREE PLANTATIONS

by

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(ABSTRACT)

Procerum root disease (PRD), caused by *Leptographium procerum* (Kendr.) Wingf., is the most serious problem facing Christmas tree growers of eastern white pine,(*Pinus strobus* L.). Limited studies have shown an association between PRD affected trees and insect infestations, and *L. procerum* has been recovered from field collected insects. The objectives of this study were to demonstrate the association of *L. procerum* with the life cycle of potential insect vectors and determine if the insect associates could transmit the fungus to healthy trees. To study the association of PRD with potential insect vectors, PRD symptomatic trees from 4 Christmas tree plantations were excavated and examined monthly, June - September in 1988 and 1989, and April - September 1990. Potential insect vectors were collected weekly in baited pit-fall traps placed in: 1) paired plots placed in asymptomatic and symptomatic areas of PRD symptomatic plantations, 2) plots in plantations where PRD was absent, 3) plots in the headlands of plantations, 4) plots in forested areas and 5) one plot in an urban setting. Trees in the plots were also inspected for evidence of weevil feeding and for development of PRD. Larvae of two weevil species, *Hylobius pales* (Herbst.) and *Pissodes nemorensis* Germ., were recovered

from 52, 42, and 43% of PRD symptomatic eastern white pine in 1988, 1989, and 1990, respectively. *Hylobius pales* and *P. nemorensis* contaminated with *L. procerum* were recovered from all plots. The proportion of *H. pales* contaminated with *L. procerum* was 73.0% in 1988, 86.5% in 1989 and 72.9% in 1990 while the proportion of *P. nemorensis* contaminated with the fungus was 17.8, 21.2 and 14.2% in 1988, 1989 and 1990, respectively. Over the three year period of the study, the proportion of PRD infected trees in the symptomatic paired plots rose from 3.6 to 29%. None of the trees in the asymptomatic plots became symptomatic. Transmission of *L. procerum* was determined by caging field collected and artificially infested *H. pales* and *P. nemorensis* on eastern white pine seedlings for 24 hours. To determine if transmission of the fungus during oviposition leads to contamination of the brood,field collected *H. pales* adults were allowed to feed and oviposit on fresh white pineee bolts. Feeding by artificially infested *H. pales* adults resulted in transmission of *L. procerum* 90 and 98% of eastern white pine seedlings in 1989 and 1990, respectively. Field collected *H. pales* adults transmitted the fungus to 58 and 68% of seedlings in 1989 and 1990, respectively. Artificially infested and field collected *P. nemorensis* adults transmitted *L. procerum* to 100 and 28% of the seedlings respectively. All bolts oviposited on by field collected *H. pales* became colonized by *L. procerum* and 100% of the weevils that emerged from them were contaminated with the fungus. The results from this study confirms the rules for insect transmission of a plant pathogen.

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Introduction and Literature Review

INTRODUCTION

Leptographium procerum (Kendr.) Wingf., the fungal cause of procerum root disease (PRD), principally affects *Pinus* species, but has also been isolated from other species including Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco), Norway spruce (*Picea abies* L.) and Fraser fir, (*Abies fraseri* (Pursh) Poir.) (Alexander *et al.* 1988). The fungus was first described by Kendrick in 1962 and named *Verticicladiella procera*, however, it has been recently reclassified into the genus *Leptographium* (Wingfield 1985). *Leptographium procerum* was first associated with mortality of eastern white pine (*Pinus strobus* L.), in 1967 (Dochinger 1967). Affected trees exhibit symptoms of delayed bud break, wilting, and girdling by a basal canker. Although previous reports have described basal cankers associated with *Leptographium* and *Verticicladiella* species on eastern and western white pine (*P. moniticola* Dougl.) (Hubert 1953; Leaphart and Gill 1959) and with a black staining root disease of eastern white pine (Gill and Andrews 1949; Leaphart 1960), there was confusion over the identification of the genera of the two pathogenic fungi.

Since the report by Dochinger (1967), *L. procerum* has been found in widely diversified habitats including landscape plantings, thinned and natural forest stands (Bertagnole *et al* 1983; Lackner and Alexander 1983; Livingston and Wingfield 1982; Sinclair and Hudler 1980), seed orchards (Webb and Alexander 1982), and Christmas tree plantations (Lackner and Alexander 1982, 1984).

The disease is responsible for mortality in Christmas tree plantations in the eastern United States (Alexander 1980; Anderson and Alexander 1979; Lackner and

Alexander 1982, 1984). In New York, forest and landscape eastern white pine up to 20 years old were damaged by *L. procerum* in six counties and the fungus was isolated from red pine (*Pinus resinosa* Ait.) in four counties (Sinclair and Hudler 1980). In Virginia, a survey of eight Christmas tree plantations revealed losses of over 700 saleable trees (6 to 10 years old) valued at $5-15 each (Lackner and Alexander 1982). Losses from this disease are not limited to Christmas trees, but mortality is observed most frequently when trees are less than 20 years old (Alexander *et. al* 1988).

GEOGRAPHICAL DISTRIBUTION AND HOST RANGE

The distribution of *Leptographium procerum* is worldwide with reports from Finland (Hallaksela 1977), Yugoslavia (Halambek 1976), New Zealand (Shaw and Dick 1980; Wingfield and Marasas 1983) and Canada (Kendrick 1962). In the U.S. *L. procerum* has been isolated from trees in New York (Sinclair and Hudler 1980), Florida, Indiana, Kentucky, Maryland, North Carolina, Ohio, Pennsylvania, South Carolina, Virginia, West Virginia (Alexander 1980) Idaho (Bertagnole *et al.* 1983), Minnesota, Michigan, Wisconsin (Wingfield 1983), Alabama, Tennessee and Mississippi (Alexander *et al.* 1988).

Although pine species are the most commonly reported hosts of *L. procerum* the fungus has been recovered from other coniferous hosts. Hosts of *L. procerum* include Jack (*P. banksiana* Lamb.), red (*P. resinosa*), ponderosa (*P. ponderosa* Laws) (Wingfield 1983); eastern white, Scotch (*P. sylvestris* L.), Austrian (*P. nigra* Arn.), (Lackner and Alexander 1982); Virginia (*P. virginiana* Mill.), shortleaf (*P. echinata* Mill.), loblolly (*P. taeda* L.), slash (*P. elliottii* Engelm.), (Horner and Alexander 1983); sand (*P. clausa* (Chapm.) Vasey) (Barnard *et al.* 1982), and lodgepole pines (*P. contorta* Dougl.) (Bertagnole *et al.* 1982). Other hosts include Douglas fir

(Harrington and Cobb 1983) and Fraser fir (Alexander *et al.* 1988). In addition, the fungus has been isolated from Norway spruce (*Piceae abies*) stumps (Hallakskela 1977).

SYMPTOMS AND DISEASE NAMES

Pines infected by *L. procerum* generally show symptoms of delayed bud break and reduction of shoot elongation followed by chlorosis and a uniform color change from yellow to reddish-brown. Visible wilting occurs in long needle species such as eastern white pine. These needles may remain attached to the tree for a year or more (Towers 1977; Anderson and Alexander 1979; Alexander 1980). A slight canker may form at the base of infected trees, accompanied by resin exudation and resin soaking of affected wood (Lackner and Alexander 1982; Sinclair and Hudler 1980; Swai and Hindal 1981). Resin exudation is often the first symptom to appear on trees with apparently healthy crowns (Alexander *et al.* 1988). These symptoms are most pronounced in the spring, but continue throughout the growing season. A basal restriction may occur on Scotch pine with a thick crust covering the area (Alexander 1980; Lackner and Alexander 1982). Removal of the bark reveals dark brown cambial discoloration of the lower stem and roots. The wood of *L. procerum* colonized roots is resin soaked (Alexander 1980; Dochinger 1967; Swai and Hindal 1981), and in cross-section, wedges of black stain are frequently evident across the sapwood zone (Shaw and Dick 1980).

Within the short period *L. procerum* has been associated with disease of conifers, there have been four common names for the disease. "White pine root decline" (Alexander 1980), "white pine wilt" (Lackner and Alexander 1982), "white pine root disease" and "procerum root disease" (Alexander *et al.* 1988). In addition, "Leptographium root decline" was proposed by Dochinger (1967) as the common

name before *L. procerum* was determined to be the causal agent of disease. "White pine root decline" and white pine root disease" by implication limit the disease to only one species, moreover, these names are often confused with "white pine decline" which is a generalized decline of white pine not associated with any one causal factor. "White pine wilt" is inadequate for describing the symptoms observed on species with short, stiff needles such as Scotch pine. Of the names proposed "procerum root disease" possesses advantages the others lack: it indicates the species of *Leptographium* involved, is general about host species, and defines the type of disease.

COLONIZATION

In the sapwood of infected trees, *L. procerum* is found in the axial and ray tracheids and ray parenchyma of the vascular system (Halambek 1976, Horner and Alexander 1985; Shaw and Dick 1976). The hyphae move from the axial tracheids and rays, and advance tangentially through bordered pit pairs (Halambek 1976; Horner 1985). The fungus advances most rapidly longitudinally and radially while tangential growth is limited. This growth pattern produces the wedge-shaped stain in cross section typical of bluestain fungi, *Ceratocystis* spp.

Bertagnole *et al.* (1983) noted that the yeast-like form of *L. procerum* allows the fungus to be carried rapidly in the sap stream and colonize tracheid and ray cells without producing dematiaceous hyphae. Yeast-like cells may occur in tracheids some centimeters in advance of mycelia.

Histological examination of thin sections of wood colonized by *L. procerum* showed that physical blockage of the tracheids to be insufficient to cause vascular dysfunction, but resin droplets were common along the walls of tracheids in black stained or resin soaked wood (Horner 1985). The accumulation of resin in the

stained and resin-soaked areas effectively blocked water movement while healthy sapwood, without an accumulation of resin, was permeable to water (Horner *et al.* 1987).

Horner *et al.* (1987) also investigated the colonization pattern of *L. procerum* in white and Scotch pine by sampling the roots, root collar and stem at selected points. *Leptographium procerum* was recovered most frequently from the root collar while the frequency of recovery declined towards either the stem or roots. Root tip infection followed by basipetal colonization could also lead to colonization of the root collar, but only three of 20 trees had a single root colonized at portions distal from the root collar which was only 2% of the 219 roots in the study. The recovery pattern of *L. procerum* in infected tissue strongly suggests that the initial colonization usually occurs in the root collar region and moves into the roots and stem.

PATHOGENICITY

Dochinger (1967) was able to kill eastern white pine seedlings by root dip inoculation with conidia of a *Leptographium* species, later identified as *L. procerum*, but gave no other details. Halambek (1976) also used root dip inoculation of eastern white seedlings with *L. procerum* and fulfilled Koch's postulates by reisolating the fungus from dead seedlings. In New Zealand, Shaw and Dick (1980) reported that inoculations with a *Leptographium* species, subsequently identified as *L. procerum*, resulted in lesions proximal to the infection point although crown symptoms were not evident one year later. However, these findings were viewed as inconclusive by many plant pathologists. Therefore, new pathogenicity tests were performed by Lackner and Alexander (1982). Two-year-old eastern white pine seedlings were inoculated with *L. procerum* by root dipping them in a conidial

suspension or by inserting colonized blocks into taproot wounds. After 10 weeks, 50% of the root dipped seedlings had died while after 12 weeks 25% of the tap root inoculated seedlings were dead. *L. procerum* was reisolated from all of the dead seedlings. A follow-up pathogenicity test conducted by Lackner and Alexander (1984) tested the pathogenicity of three different isolates of *L. procerum* to eastern white pine and loblolly pine seedlings. Seven weeks after inoculation between 85 - 100% of the seedlings were symptomatic or dead. One isolate killed significantly fewer loblolly than eastern white pine seedlings. *Leptographium procerum* was reisolated from all of the symptomatic or dead seedlings. Bertagnole *et al.* (1983) reported the pathogenicity of five *Leptographium* species using a root wound inoculation technique on lodgepole pine (*Pinus contorta* Dougl.). Resin soaking was observed in response to all species with *L. procerum* and *L. penicillatum* Grosm. producing the longest lesions.

Other pathogenicity trials have provided less definite results. Harrington and Cobb (1983) inoculated ponderosa pine and Douglas-fir seedlings with seven *Leptographium* species, including *L. procerum*. Because their isolate of *L. procerum* was unable to kill seedlings or produce any apparent disease symptoms the authors concluded that *L. procerum* was less virulent than *L. wageneri* (Kendr.) Wingf. Wingfield (1983) used toothpicks colonized by *L. procerum* to inoculate 20 eastern white pine seedlings. Because this technique did not produce mortality the author concluded that *L. procerum* was a secondary pathogen - even though this inoculation method varied radically from root-dip technique successfully used in four previous studies. In another study, Wingfield (1986) compared the virulence of *L. wageneri* and *L. terebrantis* Barras & Perry, with seven *L. procerum* isolates: five from different localities in the U. S. A., one from Yugoslavia, and one from New Zealand.

The seedlings were again inoculated using toothpicks colonized by isolates of the various fungi. The *L. procerum* isolates killed significantly fewer seedlings than the other two *Leptographium* species. Mean lesion length, measured only by discoloration, was also significantly less. The author noted the difference in mortality between this and other pathogenicity tests may have been due to the difference in inoculation technique. In the same study, one isolate of *L. terebrantis* was compared to two of *L. procerum* by making point inoculations to 15-year-old eastern white pines. The mean length of discoloration was greatest for *L. terebrantis* with the mean length of discoloration of *L. procerum* greater than the control.

Although some discrepancy exists from the results of these tests, pathogenicity is not determined merely by mortality. Discoloration may not be the best means to determine the extent of colonization by this fungus. Bertagnole *et al.* (1983) noted that the yeast-like form of *L. procerum* allows this fungus to be carried rapidly in the sap stream and colonize tracheid and ray cells without producing dematiaceous hyphae. Thus the yeast-like cells may occur in tracheids some centimeters in advance of mycelia. Wingfield (1986) considered there to be little difference in virulence between isolates, however, Lackner and Alexander (1984) reported a significant difference in mortality among their isolates. Therefore, differences in virulence among isolates as well as different inoculation techniques may have been a factor in the varying results by the different authors.

SITE AND STRESS FACTORS

Early reports of PRD noted an association between infected trees and excessive soil moisture. In Pennsylvania, Towers (1977) found diseased trees associated with shallow, clay soils with poor drainage. In a survey throughout several New York counties, Sinclair and Hudler (1980) reported infection of eastern

white pine by *L. procerum* occurred only on soils with poor drainage. The authors also found a similar occurrence on red pine. Shaw and Dick (1980) noted that the heaviest mortality associated with the fungus occurred along temporary access roads of a poorly drained stand in New Zealand. Similarly, Halambek (1981) found the disease occurred primarily on poorly-drained sites with an unsuitable air-water drainage regime.

Not all reports of PRD, however, show an association between the disease and wet sites. Swai and Hindal (1981) recovered *L. procerum* from soil beneath diseased trees on steep hillsides in West Virginia. Livingston and Wingfield (1982) recovered *L. procerum* from diseased trees from both wet sites and from sloping sites in Minnesota. *L. procerum* was the primary pathogen recovered from the roots of declining/subsoiled loblolly pine seed orchard trees growing on sandy soils (Webb and Alexander 1982). In a survey of air-pollution sensitive and tolerant eastern white pines growing along the Blue Ridge Parkway, Lackner and Alexander (1984) isolated *L. procerum* from 24% of the trees showing air-pollution injury, but from none of the tolerant trees.

From these reports it appears that trees colonized by *L. procerum* occur in a variety of sites. The variation with respect to site aspect, however, may be due to the means of dissemination of the disease. Several authors (Lackner and Alexander 1983, 1984; Wingfield 1983; Lewis and Alexander 1986; Lewis *et al.* 1987; Alexander *et al.* 1988; Raffa and Smalley 1988) have suggested that insects from the family Curculionidae, the pales weevil, *Hylobius pales* (Herbst), and the deodar weevil, *Pissodes nemorensis,* in Virginia (Lewis and Alexander 1986), and the pine root tip weevil, *H. rhizophagus* M. B. & W., and the pine root collar weevil, *H. radicus* Buch., in Minnesota (Wingfield 1983), may be vectors of *L. procerum*. Adults of all species

are soil dwellers and become active at night (Drooz 1985). Corneil and Wilson (1984a) noted that *H. pales* adults are most active above 10°C, but become less active above 30°C. During summer drought conditions the insects may be attracted to wet sites where moisture offers a more conducive micro-climate for activity. Here the insects may carry out their life cycle while inadvertently transmitting *L. procerum*. Alternately, species such as eastern white pine, which grow poorly in inadequately drained sites, may produce stress-induced volatile compounds which act as attractants to female weevils. Both *H. pales* and *P. nemorensis* oviposit by chewing niches in the inner bark of the roots and root collar (Finnegan 1958; Lynch 1984), and transmission of *L. procerum* may take place during probing by the weevils for suitable ovipositional sites. Afterwards, the weevils may move on without ovipositing, but not after having transmitted the fungus. Because the trees are off site and already stressed, mortality due to PRD may first occur in wet areas while infected trees on higher ground may take longer to succumb. Thus the association of *L. procerum* colonized trees with wet sites may be related to the activity of insect vectors rather than the ability of the fungus to infect nonstressed trees.

DISSEMINATION

Although studies of the dissemination of *L. procerum* have been undertaken, the means of spread have not been resolved. The hypotheses proposed for the spread of *L. procerum* are colonization by soilborne propagules (Lackner and Alexander 1984; Lewis and Alexander 1986) and transmission by insect vectors (Lackner and Alexander 1984; Lewis and Alexander 1986; Lewis *et al.* 1987; Raffa and Smalley 1988; Wingfield 1983).

SOIL DISSEMINATION

Pathogens that infect their host's root system by soilborne propagules usually

have the ability to survive in the soil between colonization of successive hosts. *Leptographium procerum* can remain viable for 2 years in dead white pine bark tissue (Houston 1969). Swai and Hindal (1981) recovered the fungus from 72% of soil samples collected from the rhizospheres of symptomatic eastern white pine and from 4% of asymptomatic trees using a selective medium.

Other evidence suggests that *L. procerum* does not posses the ability for long term survival in the soil in the absence of host material. Lackner and Alexander (1984) investigated survival of *L. procerum* propagules in the soil after the excavation of six PRD-symptomatic trees at two plantations in August 1980. The soil at the base of five healthy trees at the two plantations was also assayed for propagules of the fungus. Detectable levels of *L. procerum* propagules from the soil beneath the excavated PRD-symptomatic trees decreased from 3.4×10^4 and 1.7×10^4/g of soil, in August, to none in all but one of the sites by December of the same year. *L. procerum* was not recovered from soil samples collected from the base of the healthy trees. With the removal of colonized host material detectable spore survival decreased rapidly and only a small proportion of the propagules were capable of surviving as long as four months.

In an investigation of the distribution of soil-borne propagules, Lewis *et al.* (1987) found the highest proportion in the soil adjacent to the root collar ($8\text{-}12 \times 10^3$ propagules/g soil), and it decreased logarithmically towards the root tips (<40 propagules/g soil). This distribution closely reflects the colonization pattern of *L. procerum* in the roots (Horner *et al.* 1987). Soil samples taken from the root surface and at 5 and 10 cm from the root surface were not significantly different. However, the authors noted that large numbers of propagules in the soil corresponded with frequent recovery of *L. procerum* from adjacent root tissue and they concluded that

frequent recovery of *L. procerum* from adjacent root tissue and they concluded that the propagules originated from the colonized roots. Infection of white pine seedlings by *L. procerum*, planted at varying distances from diseased trees, was related to their proximity to the root collar of the diseased trees rather than the presence of detectable levels of the fungus in the soil. Thus the source of *L. procerum* inoculum in the soil was the root collar and the proximal roots of diseased trees (Lewis *et. al* 1987).

INSECT ASSOCIATIONS

The conidiophores of *L. procerum* like other bluestain fungi produce sticky, mucilagenous spores which are ill suited to wind, water-splash, or soil-borne dispersal, but are ideally suited to dispersal by insects. Bluestain fungi and wood-inhabiting arthropods often share common habitats (Dowding 1984). In wind tunnel experiments, Dowding (1969) noted that spore dispersal of fungi with sticky spores, such as *L. procerum*, was favorable only in hydrophilic mists or by splashing water. He concluded that the likely means of dispersal of such species was by splash drops or insects, but not by air currents.

The vector hypothesis of the dissemination of *L. procerum* is supported by the observations of several authors (Lackner and Alexander 1983, 1984; Wingfield 1983; Lewis and Alexander 1986; Lewis *et al.* 1987; Raffa and Smalley 1988). Procerum root disease infected trees generally occur randomly among uninfected trees without a distinct disease center characteristic of Phytophthora root rot. *Leptographium procerum* is most likely an anamorph of an *Ophiostoma* (*Ceratocystis*) species (Horner 1985) and the association between insects and *Ophiostoma* (*Ceratocystis*) bluestain fungi has been well established (Bakshi 1951; Findlay 1959;

Hunt 1956; Mathre 1964; Verral 1941). The association between insects and *Leptographium* species has been documented by Harrington (1988).

Insect galleries have been observed adjacent to stain margins of *Leptographium wageneri* (Landis and Helberg 1976) and both the imperfect and perfect stages of the fungus have been found occurring together only in insect galleries of diseased roots (Goheen and Cobb 1978). Witcosky and Hansen (1985), found a close association between *L. wageneri* infected trees and three root colonizing insects: *Hylastes nigrus* (Mann.) (Scolytidae), *Steremnius carinatus* (Boh.) (Curculionidae) and *Pissodes fasciatus* LeC. (Curculionidae). Harrington *et al.* (1985) caged field collected *H. nigrus* adults and *H. nigrus* adults artificially contaminated with *L. wageneri* on Douglas-fir seedlings. After three months, one of 47 seedlings caged with field collected *H. nigrus* and three of 22 seedlings caged with artificially contaminated insects showed symptoms of disease. Witcosky *et al.* (1986) confirmed the ability of *H. nigrus* to vector *L. wageneri* to Douglas-fir, and also demonstrated the ability of two root feeding weevils, *Pissodes fasciatus* and *Steremnius carinatus* to transmit the fungus.

Leptographium terebrantis, is associated with at least three insect species and has been isolated from the roots of dying pines (Highley and Tatter 1985; Wingfield 1983). Bark beetles associated with this fungus most often attack stressed trees (Baker 1972) and *L. terebrantis* may contribute to the death of the insect-infested trees (Wingfield *et al.* 1988). Unnaturally high rates of mortality of Japanese pine (*Pinus thunbergiana* Franco) are thought to be caused by infestation of the bark beetle, *Dendroctonus terebrans* (Oliv.) and concomitant infection by *L. terebrantis* (Highley and Tatter 1985). Pathogenicity of *L. terebrantis* was verified by Wingfield (1986) and Rane and Tatter (1987), with the latter investigating the pathogenicity of

this fungus on Japanese black pine seedlings. On native pine hosts colonization is generally restricted to the vicinity of insect galleries (Harrington and Cobb 1983; Harrington 1988).

The possible dissemination of *L. procerum* by insect vectors is supported by the observations of several authors who noted an association between the fungus and root feeding insects, principally weevils - *Hylobius pales*, and *Pissodes nemorensis* in Virginia (Lackner and Alexander 1983, 1984; Lewis and Alexander 1986; Lewis *et al.* 1987), and *H. pales*, *H. radicus* and *Pachylobius picivorus* in Minnesota and Wisconsin (Wingfield 1983; Raffa and Smalley 1988). Vectors may contact inoculum in *L. procerum* infected brood trees and then disperse to feed on healthy, wounded, or stressed trees.

In a survey of ozone sensitive pine along the Blue Ridge Parkway, Lackner and Alexander (1983) recovered *L. procerum* from the roots along with the galleries, pupal chambers and adult insects of two weevil species, *Hylobius pales* and *Pissodes nemorensis*. In a second study Lackner and Alexander (1984) found the conidia of *L. procerum* in insect galleries and they recovered three bark beetle genera, *Pityokteines* Fuchs, *Pityogenes* Bedel. and *Pityophthorus* Eich., and one weevil species, *P. nemorensis*, from the stems of PRD-symptomatic trees. In Wisconsin, Wingfield (1983), isolated *L. procerum* from trees infested by several weevil species including *H. radicus, H. pales*, *P. nemorensis*, and *Pachylobius picivorus* (Germ.).

Lewis and Alexander (1986) showed that *H. pales* and *P. nemorensis* carry the fungus in the field. They surveyed ten eastern white pine Christmas tree plantations for insects over a four week period using pit-fall traps baited with split bolts of eastern white pine. The number of plantations was equally divided between those with PRD-symptomatic trees and plantations where no PRD-symptomatic trees

were present. Insects were found carrying *L. procerum* in both symptomatic and asymptomatic plantations. Insects recovered included weevils, *Hylobius pales* and *Pissodes nemorensis*, and bark beetles of the genera, *Pityogenes*, *Orhtotomicus* Ferr., *Xyleborus*, and *Hylastes* Eich. Over 64% of the weevils were contaminated with *L. procerum*, but less than 1% of the bark beetles carried the fungus. In addition, the ability of the weevils to transmit *L. procerum* to fresh eastern white pine bolts was demonstrated. However, the authors did not distinguish between weevil species carrying *L. procerum* in the field, or which species were able to transmit the fungus to the bolts.

Christmas tree plantations offer an ideal location for weevils such as the pales and deodar weevils to complete their life cycles (Anderson 1980; Corneil and Wilson 1984a, 1984b; Finnegan 1956, 1958, 1959; Lynch 1984; Mosher and Wilson 1977).

Hylobius pales. The pales weevil is distributed throughout the eastern half of the United States and southeastern Canada. In the United States its range extends from Florida to Maine and west to Minnesota and eastern Texas. The host range of *H. pales* includes 29 species in 11 genera, but most damage occurs on the genus *Pinus* (Lynch 1984).

In the northerly regions of North America, *H. pales* has only one generation or a partial generation annually while in the south there is one complete generation with a partial overlapping second one annually (Lynch 1984, Drooz 1985). Both larvae and adults overwinter in all regions (Lynch 1984). Adult females oviposit in the bark of roots and stems of stumps or weakened trees. Anderson (1980) showed that there is one complete generation with a partial overlapping second one in

Virginia Christmas tree plantations. Seasonal activity was greatest for *H. pales* from April through July.

Feeding by adult *H. pales* has been recorded on new shoots and one year old bark of mature trees, however, it is the damage they cause to seedlings in reforested areas that has been of greatest concern to foresters (Lynch 1984; Drooz 1985). Characteristically, bark is chewed off in irregular patches leaving ragged, irregular pits in the bark exposing the wood (Finnegan 1958). Light feeding results in the production of oleoresin and the wounds heal, but continued feeding may result in the girdling and death of the seedling. Feeding preference tests by Hunt and Farrier (1974) indicated that field-collected weevils preferred twig bark, upper tree bark, and root bark to seedling bark. They attributed the nonpreference for seedling bark to errors in experimental design or lack of preference and concluded that seedlings may be less preferred but a more available food source.

In Christmas tree plantations, *H. pales* congregate near fresh stumps in the spring and move to nearby trees in the summer (Corneil and Wilson 1984a). During the day, they remain at the base of their host tree and move onto the tree after dark. The weevils are most active above 11^{o} C and below 30^{o} C. Although they are strong fliers, once they find a suitable habitat they walk and rarely fly, even under near fatal conditions (Corneil and Wilson 1984b). The major impact of *H. pales* activity in Christmas tree plantations has been seedling mortality and cosmetic damage, caused by feeding on the new growth of marketable trees (Corneil and Wilson 1980, 1986). In Virginia, Anderson (1980) concluded that infestations by *H. pales* and *P. nemorensis* may result in significant mortality of newly planted seedlings.

***Pissodes nemorensis*:** The biosystematics of *Pissodes nemorensis* and *P. approximatus* have been recently revised by Phillips *et al.* (1987) who proposed the conspecificity of the two species. Thus *Pissodes approximatus* Hopkins (1911) is a junior synonym of *P. nemorensis* Germar (1824) and a new common name, the eastern pine weevil, has been approved for the combined species (Stoetzel 1989). The range of *P. nemorensis* extends from Nova Scotia to Manitoba in Canada, and southward from Minnesota to the southern states in the United States. The host range of *P. nemorensis* primarily includes members of the genus *Pinus* and *Piceae.*

In northern regions the life cycle may be completed in one or two years while in southern regions there is one generation per year (Drooz 1985). Females deposit eggs in pockets chewed through the bark of recently cut stumps and logs, and on the main stems or branches of dead and dying trees. In Virginia Christmas tree plantations, Anderson (1980) found one complete generation per year. Eggs laid in the spring resulted in emergence of adults from the stumps in July and August of the same year. No larvae or pupae overwintered in the stumps. Seasonal activity of adults was greatest from March through June.

When feeding, *P. nemorensis* adults leave the outer bark intact except for small puncture holes through which they inserts their beak and chew out larger areas of the inner bark. Feeding has been reported on the bark of new shoots, branches, stems and roots of seedlings, and mature trees (Finnegan 1958).

Finnegan (1956) reported that damage caused by large populations of the eastern pine weevil in Christmas tree plantations may result in the reduction and quality of trees where feeding is heavy, and in some instances even tree mortality. However, like the pales weevil, *P. nemorensis* is most noted for killing seedlings in Christmas tree plantations and in reforested areas (Drooz 1985).

To be classified as a vector insects must satisfy Leach's postulates for insect transmission of a plant disease. The insects must: a) be constantly associated with diseased trees; b) visit healthy trees under conditions suitable for transmission; c) carry inoculum in the field; and d) transmit the pathogen under laboratory and field conditions (Leach 1940). Although the above weevil species have been suggested as vectors of *L. procerum* no study has demonstrated the ability of these weevils to transmit the pathogen to living trees. Thus at this time the etiology of PRD is incomplete.

MANAGEMENT AND CONTROL

The principal studies into PRD have been conducted in Christmas tree plantations, therefore the strategies for managing the disease are also directed to this area. The following are the recommended control measures: a) plant trees on sites suitable for the species, and avoid planting white or Scotch pine on sites prone to flooding or drought, b) control weevils and bark beetles with insecticide sprays and cultural practices, c) remove diseased trees and their root systems, as well as, slash from within and around the plantation, d) do not replant white pine in infested areas, and e) keep weeds under control by mowing or with herbicides. (Anderson and Alexander 1979; Alexander 1980; Alexander *et al.* 1988).

RESEARCH OBJECTIVES

Procerum root disease caused by *Leptographium procerum* is presently the most serious problem to eastern white and Scotch pines faced by Virginia Christmas tree growers. Research over the last 15 years has determined the pathogenicity, host and geological ranges, colonization patterns in infected wood, and some site and biotic factors associated with the disease. In addition, conclusive evidence has been shown that PRD is not soil-borne. Insects, principally *H. pales* and *P.*

nemorensis, have been proposed as vectors of *L. procerum*, but all of Leach's Postulates for insect transmission of a plant pathogen have not been fulfilled.

The objectives of this research were:

1. To determine the association of *L. procerum* with the life cycle of potential insect vectors and whether the species, numbers and percent of insects carrying *L. procerum* inoculum are similar: a. between the symptomatic and asymptomatic areas of the plantation,
   b. throughout the growing season,
   c. between species.
2. Establish experimentally the ability of *Hylobius pales* and *Pissodes nemorensis* to transmit *L. procerum* to eastern white pine.
3. To evaluate control techniques to limit the spread of *L. procerum* in Christmas tree plantations.

Chapter II

The Association of Root Feeding Insects With Procerum Root Disease

INTRODUCTION

The epidemiology of procerum root disease (PRD) was studied by Lackner and Alexander (1984) who observed the distribution of diseased trees in two plantations over a 14 month period. During this time, tree mortality was 34% in the first plantation and 17% in the second. *Leptographium procerum* was isolated from soil samples taken from the area of diseased trees. In addition, the stems of diseased trees were infested by insects including bark beetles and weevils. However, no conclusive evidence for the mechanism of pathogen spread was presented.

Lewis and Alexander (1987) showed that *L. procerum* was not soil-borne and in a second study examined the role of insects as possible vectors of *L. procerum* (Lewis and Alexander 1986). The authors surveyed ten eastern white pine Christmas tree plantations for four weeks using pit-fall traps baited with split bolts of eastern white pine. Insects recovered included weevils, *Hylobius pales* and *Pissodes nemorensis*, and bark beetles, *Pityogenes* sp., *Orhtotomicus* sp., *Xyleborus* sp., and *Hylastes* sp. In all plantations they found 64% of the weevils to be contaminated with *L. procerum*, but less than 1% of the bark beetles. The authors also demonstrated the ability of weevils to transmit *L. procerum* to fresh pine bolts. Although Lewis and Alexander (1986) were able to find a relationship between two weevil species and *L. procerum*, their study was completed over a short period of the growing season. Moreover, no distinction was made between the species contaminated with *L. procerum* or their distribution within the plantation.

The present study was undertaken over a three year period, from April to September each year when insects are most active in Christmas tree plantations

(Anderson 1980). The objectives of the study were to establish the association of *L. procerum* with potential insect vectors, especially *Hylobius pales* and *Pissodes nemorensis*, to determine whether the species, numbers, and percent of insects carrying *L. procerum* inoculum were similar between species, the symptomatic and asymptomatic areas of the plantation, throughout the growing season, and to observe the rate of disease progress in PRD-symptomatic plantations. This test would confirm or deny the second and third of four laws proposed by Leach (1947) for identifying insect vectors: vectors carry inoculum in the field.

MATERIALS AND METHODS

Insect Survey. In 1988, 25 circular, fixed-radius plots (radius 5.65 m, area = 1/100 ha) were set out in 10 Christmas tree plantations, located in three counties in southwestern Virginia (Appendix I). Of these, 5 plots were in three plantations with no history PRD. The other 20 plots were placed in seven plantations where PRD-symptomatic trees were present, as paired plots: an area with symptomatic trees was paired with an asymptomatic area with trees of the same size. Three large plantations were subdivided into smaller 15-ha "plantations". Each of the symptomatic plots had at least one PRD-symptomatic tree inside the plot and two symptomatic trees within 50 m of the plot center. Asymptomatic plots were placed in areas of the plantation where there were no symptomatic trees within a 50 m radius of the plot center. Five of the paired plots were placed in areas where harvest had occurred during the winter of 1987 - 1988. In plantations with no PRD-symptomatic trees, the plots were placed in areas where no previous harvest had occurred. All plots were at least 25 m inside the plantation boundary.

To confirm the presence or absence of *L. procerum* in the area of the plots, increment cores were taken from base of 20 trees outside of the plot perimeter. At

symptomatic plots, preference was given to trees showing symptoms of PRD. Samples of wood and bark tissue were removed from opposite sides of the of the root collar and plated onto 1.5% malt agar amended with 500 mg/L actidione (AMA) (McCall and Merrill 1980). The plates were observed for colonies of *L. procerum* after 14 days.

Pit-fall traps containing split-bolts of eastern white pine, designed to attract root feeding insects (Taylor and Franklin 1970), were placed at the center of each plot. Each trap consisted of a pit 10 cm deep and 30 X 45 cm, and contained 6 bolts from branches of healthy eastern white pine 2 - 6 cm in circumference and 25 cm long, split longitudinally. Pits were placed, where possible, on sloping ground and dug with an incline to avoid filling with rainwater. Fresh pine boughs were placed over the pit to shade the bolts. The pine bolts were collected and replaced weekly. The bolts and bottom and sides of the pit were closely examined for weevils. Collected weevils were placed in 40 x 15 mm petri plates and sealed with masking tape, placed in plastic bags with the bolts and removed to the laboratory. The traps were operational for 24 weeks, mid-April to the end of September, in 1988 and 1989, and 28 weeks, mid-March to the third week of September, in 1990.

At the laboratory, the weevils were picked up with flame-sterilized forceps and individually placed on AMA plates for 24 hr. The bolts were carefully debarked and any bark beetles in the bark were individually placed onto AMA plates with sterilized forceps. For each insect the date, plot, weevil species or the number of bark beetles were recorded. After removal from the petri plates the weevils were placed in 30 x 19 x 9 cm plastic boxes together with fresh pine twigs and reserved for further experiments. Representative samples of bark beetles and weevils were placed in 70% EtOH and reserved for species verification. Bark beetles were

identified to family at the Insect Identification Laboratory, VPI & SU, Blacksburg, VA and by Dr. D. M. Anderson, Systematic Entomology Laboratory, Plant Sciences Institute, USDA. Positive identification of *Hylobius pales* and *Pissodes nemorensis* was made by Dr. Tom Atkinson, Department of Entomology and Nematology, University of Florida, Gainesville, FL. Insect isolation plates were observed for the presence of *L. procerum* after incubation at 20^{o} C for 14 days.

In 1990 an additional 10 plots were established. One plot was in an urban setting, in Blacksburg VA, where a number of 20-year-old eastern white pines had succumbed to PRD over a 4 year period. Six plots were placed in eastern white pines stands adjacent to plantations used in the study. Three other plots were established in naturally regenerated mixed stands of eastern white pine and Virginia pine one-half kilometer from the nearest Christmas tree farm.

Data Analysis. The difference in numbers of each weevil species and the numbers carrying *L. procerum* between symptomatic and asymptomatic plots were analyzed using one-tailed, paired *t*-tests on SYSTAT version 4.1 (Systat Inc). The difference in numbers between weevil species and numbers carrying *L. procerum* within symptomatic and asymptomatic plots, in asymptomatic plantations, and in plots outside plantation boundaries were analyzed using one-tailed, *t*-tests on SYSTAT version 4.1 (Systat Inc). Between year changes in the total number of weevils in the symptomatic and asymptomatic plots, and the asymptomatic plantations were analyzed using a one-way ANOVA on SYSTAT version 4.1. Between year differences in the percentage of *H. pales* contaminated with the fungus in the symptomatic plots, asymptomatic plots and in the asymptomatic plantations were analyzed using the Kruskal-Wallis test for tied ranks SYSTAT version 4.1.

Disease development. The plots with the pit-fall traps were also used to monitor disease development in the plantations. In each plot, the disease status of all trees within the plots was recorded. The presence or absence of *L. procerum* was confirmed in the PRD-symptomatic plots described above. To establish a baseline when symptom development began, symptomatic trees within the PRD-symptomatic plots were not considered and only apparently healthy trees at the time of plot establishment were utilized. As trees within the plot boundary became symptomatic of PRD they were flagged with survey ribbon and the date that symptoms were first detected was recorded. Trees within all plots were examined on a monthly basis for signs of weevil feeding near the root collar. Such trees were also flagged and the date noted. At the end of the collection period each year, bark and sapwood samples from the area fed on by the weevils were collected. At the laboratory, the samples were aseptically plated onto AMA and observed after three weeks for conidia of *L. procerum*.

RESULTS

In the PRD-symptomatic plantations, the presence of *L. procerum* in symptomatic plots was confirmed by the recovery of the fungus from increment cores. *L. procerum* was not isolated from any of the cores taken from asymptomatic plots and none of the trees within the asymptomatic plots became infected with PRD. During the study, several trees at one asymptomatic plantation began showing symptoms of PRD and *L. procerum* was isolated from two of the symptomatic trees. PRD-symptomatic trees did not appear at the other asymptomatic plantations.

Insect survey. Weevil species collected included, *Hylobius pales, Pissodes nemorensis* and *Otiorhynchus rugostriatus* (Geoze). *Otiorhynchus rugostriatus*, the

rough strawberry weevil, was not considered after 1988 as none of the insects collected were contaminated with *L. procerum* and the insect is not known to feed or reproduce on pines (Johnson and Lyon 1988). The bark beetle species collected were from families associated with dead or dying trees and not recorded as the primary means of tree death (Drooz 1985). Genera included: *Pityogenes* Bedel, *Orthotomicus* Eich., *Ips* Degeer and *Pityophthorus* Eich. Bark beetles identified to species included *Pityogenes hopkinsi* Swaine, *Ips pini* (Say), and *Orthotomicus caelatus* Eich.

Insects contaminated with *L. procerum* were collected from all plots, including those in plantations where no PRD-symptomatic trees were present. In 1990, four weevils, two *H. pales* and two *P. nemorensis* were collected in the trap placed in an urban setting and one of each species carried *L. procerum*. The total trap catch inside all the plantations was 366 weevils and 259 bark beetles in 1988, 323 weevils and 111 bark beetles in 1989, and 188 weevils and 134 bark beetles in 1990. The total number of *H. pales* trapped in all plantations was 163 in 1988, 252 in 1989, and 105 in 1990 while there were 180, 71, and 83 *P. nemorensis* collected in 1988, 1989, and 1990, respectively. Twenty three *O. rugostriatus* were collected in 1988. In addition, 112 weevils, 89 *H. pales* and 23 *P. nemorensis*, and 8 bark beetles were collected in traps outside of the plantations in 1990.

Leptographium procerum was the most consistently recovered pathogen from all insects in this study (Tables 1 - 3). Other pathogens recovered included *Leptographium serpens* (Goid.) Siem., *Ophiostoma piceae* (Munch) Sydow & Sydow, *Ophiostoma ips* (Rumb.) Nannf., *Ceratocystis sagmatospora* Wright & Cain, and a *Graphium* spp. (see Appendix I). The overall proportion of *H. pales* contaminated with *L. procerum* was 73.0% in 1988, 86.5% in 1989, and 72.9% in 1990 while *P.*

nemorensis carried 17.8, 21.2 and 14.2% in 1988, 1989 and 1990, respectively. For bark beetles the overall proportion contaminated with *L. procerum* was 2.7% in 1988 and 1989, and 10.8% in 1990. In 1990, 5 *P. hopkinsi*, and two *O. caelatus* were positively identified, and found carrying *L. procerum*. This is the first report of these bark beetle species carrying *L. procerum*.

The mean number of weevils caught in the different plot types and the percentage contaminated with pathogenic fungi are shown in Tables 1 - 3. The species distribution and the numbers contaminated with *L. procerum* remained constant between symptomatic and asymptomatic areas of symptomatic plantations. There was no significant difference in the number of *P. nemorensis* or the numbers carrying *L. procerum* between symptomatic and asymptomatic plots (Table 1). In all three years, *H. pales* were found in significantly greater numbers in symptomatic plots than asymptomatic plots and significantly more *H. pales* were contaminated with *L. procerum* in symptomatic plots than those in asymptomatic ones. In the symptomatic plots, there were significantly more *H. pales* than *P. nemorensis* and significantly more *H. pales* were contaminated with *L. procerum*. There was no significant difference in the number of *H. pales* and *P. nemorensis* trapped in the asymptomatic plots. In 1989, *H. pales* in the asymptomatic plots carried significantly more *L. procerum* than *P. nemorensis*, but not in the other two years .

The total number of *H. pales* within the symptomatic plots did not vary significantly among years, nor did the percentage carrying *L. procerum* (Table 1). In the asymptomatic plots, the total number of *H. pales* also did not vary significantly among years, nor did the percentage carrying *L. procerum* (Table 1). The mean number collected of each weevil species in individual plots did vary (Figs. 1 - 3),

suggesting that the relative attractiveness and/or suitability of host material at these sites was changing over the period of the study.

The total numbers of *P. nemorensis* in the symptomatic plots varied significantly ($p < 0.05$) among years with 1988 > 1990 > 1989 (Table 1). However, the percentage carrying *L. procerum* was not significantly different between years. In the asymptomatic plots there were significantly more ($p < 0.05$) *P. nemorensis* in 1988 than in 1989 or 1990, but the percentage contaminated with *L. procerum* was not significantly different among years (Table 1).

In the asymptomatic plantations there were significantly more ($p < 0.05$) *P. nemorensis* than *H. pales* in 1988, but the numbers of the two species did not vary significantly from each other during the next two years (Table 2). There was also no significant difference between the numbers of *H. pales* and *P. nemorensis* carrying *L. procerum* each year. The total number of *H. pales* or *P. nemorensis* collected did not vary significantly between years. However, the percentage of *H. pales* carrying the fungus was significantly higher (Kruskal-Wallis $p = 0.05$) in 1989 than the other years. The percentage of *P. nemorensis* carrying the fungus was not significantly different among years.

The composition and numbers of insects collected outside the plantation boundaries in 1990 was similar to those found in the symptomatic plots in PRD-symptomatic plantations (Tables 1 and 3). Significantly more ($p < 0.05$) *H. pales* were collected than *P. nemorensis*, and significantly more ($p < 0.05$) *H. pales* were contaminated with *L. procerum* than *P. nemorensis*.

The seasonal distribution of weevil species in the plantations is shown in Figs. 4 - 6. In 1988, the peak trap catch for both species began in mid-May while in 1989 the peak activity of *P. nemorensis* began earlier and the activity of *H. pales*

occurred three weeks later than in 1988. In 1990, weevil activity was not as pronounced as the previous two years and there were no distinct peak activity periods for either species (Figs. 4 - 6). However, the activity of *H. pales* in the plots outside the plantations did show a definite peak in 1990 (Fig. 7), beginning in late March, which was much earlier than that inside the plantations in 1988 and 1989. The activity of the weevils outside the plantations, however, did not occur earlier than that inside the plantations. The activity of *P. nemorensis* outside the plantations in 1990 did not appear to differ from those in the plantations.

The monthly variation in the percentage of both weevil species carrying *L. procerum* in the symptomatic and asymptomatic plots is shown in Fig. 8. In the symptomatic plots, monthly means of weevils carrying *L. procerum* were not significantly different from the yearly means for 1988 and 1989 ($p < 0.05$, X^2 test). However, the 1990 monthly means were significantly different from the yearly mean for 1990. The monthly means for *H. pales* carrying *L. procerum* in the asymptomatic plots were significantly different from the yearly mean for all years as were the means for *P. nemorensis*. Thus for 1988 and 1989, the proportion of *H. pales* carrying *L. procerum* within the symptomatic plots was constant during the collection period. In 1990, the proportion of *H. pales* in the symptomatic plots was not constant throughout the collection period. The proportion of *P. nemorensis* carrying *L. procerum* in the symptomatic plots and the proportion of either species carrying the fungus in the asymptomatic plots was not constant throughout the collection period during any year of the study.

Disease development. A total of 253 apparently healthy trees were counted in symptomatic plots in April, 1988. Of these, 9 showed symptoms of PRD by October, 1988. The following year 14 others became PRD-symptomatic and in 1990

another 41 trees became symptomatic. A total of 35 trees were harvested from all plots in 1988 and 56 in 1989. The proportion of all trees remaining in the plots after harvest each year and those infected by the disease is shown in Fig. 9. The numbers of trees lost to the disease was not uniform between the symptomatic plots and losses ranged from 0 - 68%.

During the study period, 9 trees within the plots of three plantations had evidence of weevil feeding at the root collar which conformed with the description of *H. pales* feeding by Finnegan (1958). Two trees with weevil feeding were found in a PRD-symptomatic plot in April 1989, but *L. procerum* was not recovered from these trees. Both trees were harvested as Christmas trees the following winter so the trees could not be observed for symptoms of PRD. In March, 1990, 7 trees were observed with evidence of weevil feeding, three in two PRD-symptomatic plots, and 4 in the same PRD-asymptomatic plantation. *Leptographium procerum* was recovered from two of the trees in the PRD-symptomatic plots, and from two of 4 trees in a plot in the asymptomatic plantation.

DISCUSSION

The results of this study confirms the transport of *L. procerum* by *H. pales* and *P. nemorensis*, and provide evidence for the second and third of four postulates proposed by Leach (1940) for identifying insect vectors of plant pathogens which are: insects regularly visit healthy plants under conditions suitable for the transmission of the pathogen, and insects carry *L. procerum* inoculum in the field (Tables 1 - 3). The frequency of fungus transport was > 72% for *H. pales*, > 14% for *P. nemorensis* and > 3% for bark beetles during the three years of the study. Weevils carrying *L. procerum* were recovered from every plot in PRD-symptomatic and asymptomatic plantations, in forested areas outside the plantations, in the forest

and at one site in an urban setting. Evidence of *H. pales* feeding was observed on nine trees in three plantations during the study. Further evidence demonstrating the recovery of *L. procerum* with weevil feeding is provided in Chapter III.

Lewis and Alexander (1986) reported 64% of weevils, *H. pales* and *P. nemorensis*, in their survey were contaminated with *L. procerum*. In another study, 48% of *H. pales* in Wisconsin and 50% of *H. pales* in Michigan, carried the fungus (Wingfield 1983). These studies combined with the present one suggest that the transport of *L. procerum* by *H. pales* may be a common phenomenon in the eastern United States.

This study detailed the species, numbers, proportion contaminated with pathogenic fungi, and distribution of insects within PRD-symptomatic and asymptomatic plantations. *L. procerum* was the most consistently recovered pathogen from all the insects, and *H. pales* was the insect most frequently collected and the one most frequently contaminated with the fungus (Tables 1 - 3). In symptomatic plantations, the numbers of *H. pales* were always significantly higher in symptomatic than asymptomatic plots. *Hylobius pales* collected in symptomatic plots carried significantly more *L. procerum* than either *H. pales* or *P. nemorensis* found in asymptomatic plots. In symptomatic plots the yearly total and percentage of *H. pales* contaminated with *L. procerum* did not vary significantly between years while both the total number and proportion of *P. nemorensis* carrying the fungus varied significantly all three years. Moreover, in all plantations the percentage of *H. pales* contaminated with *L. procerum* remained higher than that of *P. nemorensis* throughout the growing season (Fig 8). These results combined with the insect's ability to transmit *L. procerum* to eastern white pine seedlings and 5-year-old trees

(Chapter IV), and the recovery of the fungus from weevil feeding mentioned above and in Chapter III, suggest that *H. pales* is the most important vector of PRD.

The high number of trees infected in the plots in 1990 was reflected in the plantation area outside the plots, and in farms throughout southwestern Virginia. The estimated number of eastern white pine lost to PRD was over 800,000 trees (S. A. Alexander personal communication).

Table 2.1. The mean number of insects (±SE) and the percentage contaminated with *L. procerum* recovered from baited pit-fall traps in 10 paired plots in symptomatic and asymptomatic areas of procerum root disese symptomatic plantations, during 1988 - 1990. .

Plot type	Insect species					
	H. pales		*P. nemorensis*		Bark beetles	
	X±S.E.	*L. procerum* (%)	X±S.E.	*L. procerum* (%)	X±S.E.	*L. procerum* (%)
1988[1]						
Symptomatic	9.8±1.8[a3]	79.6	4.9±1.0	14.3	15.6±4.6	3.8
Asymptomatic	5.1±1.8[b]	62.7	8.5±1.5	21.2	8.0±3.7	0
1989[1]						
Symptomatic	15.2±4.4[a]	90.8	1.4±0.4	42.9	3.6±1.6	0
Asymptomatic	6.2±2.0[b]	82.3	2.9±0.7	10.3	3.4±2.5	2.9
1990[2]						
Symptomatic.	7.7±2.2[a]	77.9	2.7±0.7	20.9	5.4±2.8	20.8
Asymptomatic.	2.1±0.9[b]	71.4	3.3±0.7	15.2	4.6±2.5	4.3

1 survey period 24 weeks

2 survey period 28 weeks

3 Different superscripts, within years, indicate significantly different means at P = 0.05. Paired t-test (one-sided) used for all comparisons.

Table 2.2. The mean number of insects (±SE) recovered from baited pit-fall traps and the percentage contaminated with *L. procerum* from 5 asymptomatic plantations in 1988 and 1989, and 4 asymptomatic plantations in 1990.

	Insect species					
	H. pales		*P. nemorensis*		Bark beetles	
YEAR	X±S.E.	*L. procerum* (%)	X±S.E.	*L. procerum* (%)	X±S.E.	*L. procerum* (%)
1988[1]	2.8±2.1	64.3	9.2±4.1	15.2	4.6±1.9	4.3
1989[2]	7.6±2.2	76.3	6.0±2.0	22.5	5.2±4.6	4.9
1990[3]	1.8±1.5	57.1	4.6±2.2	11.1	9.6±4.8	6.9

1 survey period 20 weeks
2 survey period 24 weeks
3 survey period 28 weeks

Table 2.3. Mean number of insects (±SE) recovered in 1990 from baited pit-fall traps and the percentage contaminated with L. procerum in individual plots randomly placed in the headlands of 6 Christmas tree plantations, in 3 mixed eastern white and Virginia pine stands, and one plot in an urban setting placed where 20-year-old eastern white pine had been killed by procerum root disease.

Plot type	Insect species					
	H. pales		P. nemorensis		Bark beetles	
	X±S.E.	L. procerum (%)	X±S.E.	L. procerum (%)	X±S.E.	L. procerum (%)
Headlands	12.2±5.1	72.6	3.7±1.5	4.5	0.8±0.7	20.0
Pine stands	5.3±1.3	68.8	0.3±0.3	0	1.0±0.6	0
Urban setting	2.0	50.0	2.0	50.0	11.0	0

Fig. 2.1. Mean number of *H. pales* recovered per plot, per week in a) symptomatic and b) asymptomatic areas of PRD-symptomatic plantations, 1988 - 1990.

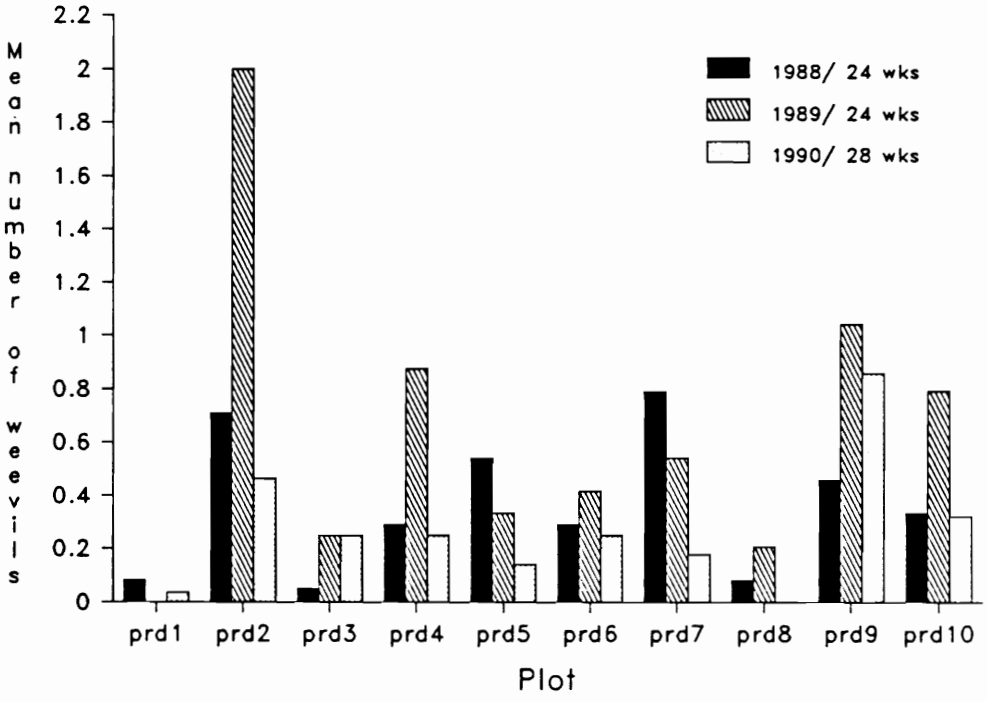

Fig. 2.1a.

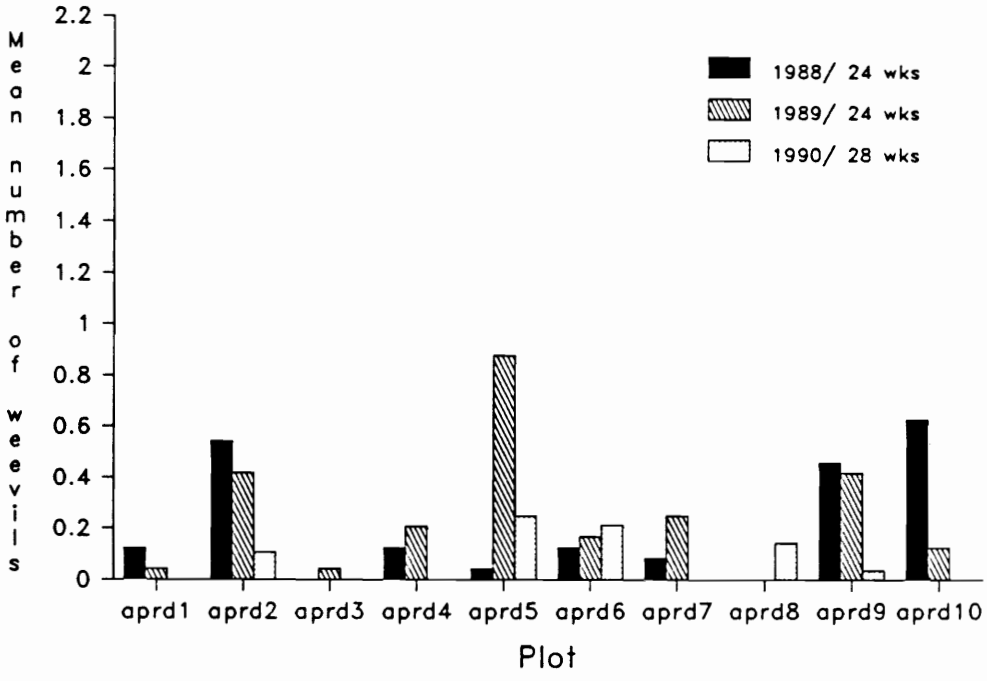

Fig. 2.1b.

Fig. 2.2 Mean number of *P. nemorensis* recovered per plot, per week, in a) symptomatic and b) asymptomatic areas of PRD-symptomatic plantations, 1988 - 1990.

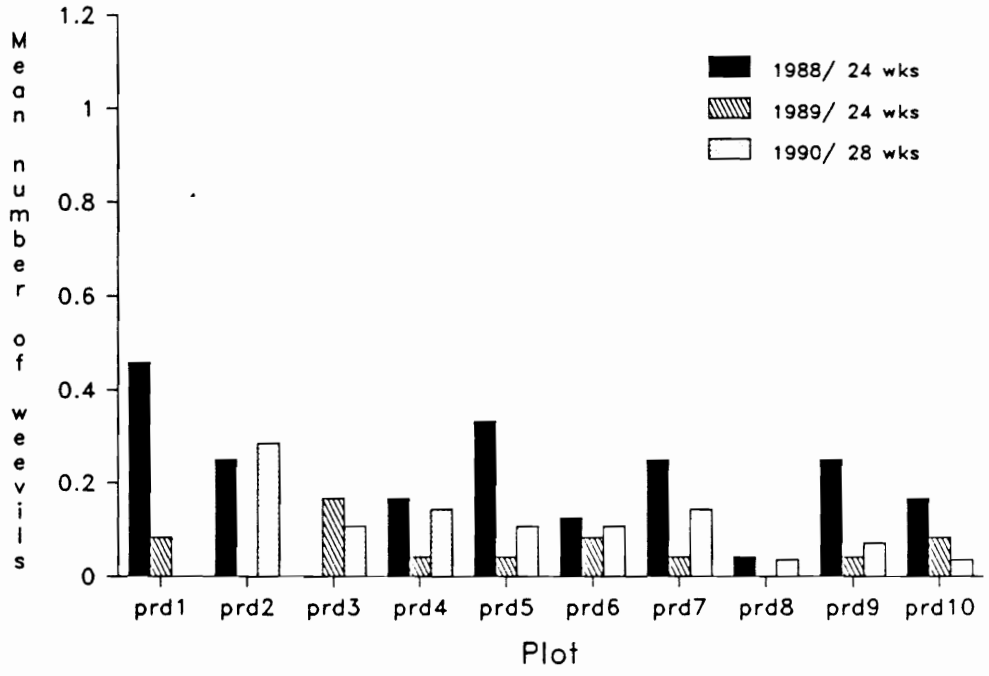

Fig. 2.2a.

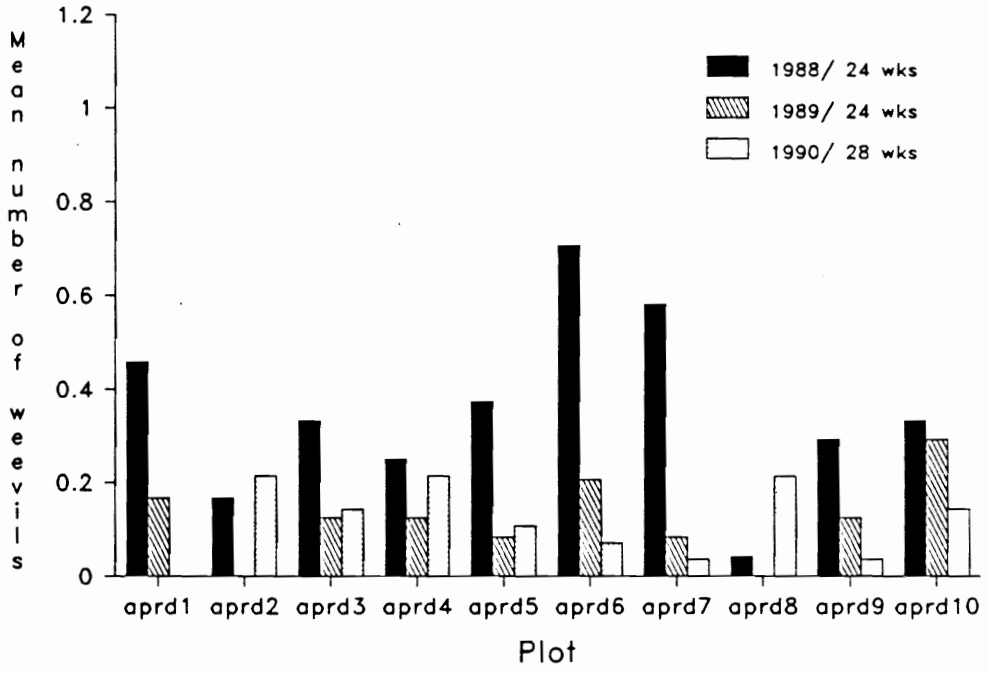

Fig. 2.2b.

Fig. 2.3. Mean number of a) *H. pales* and b) *P. nemorensis* recovered per plot in PRD-asymptomatic plantations, 1988 - 1990.

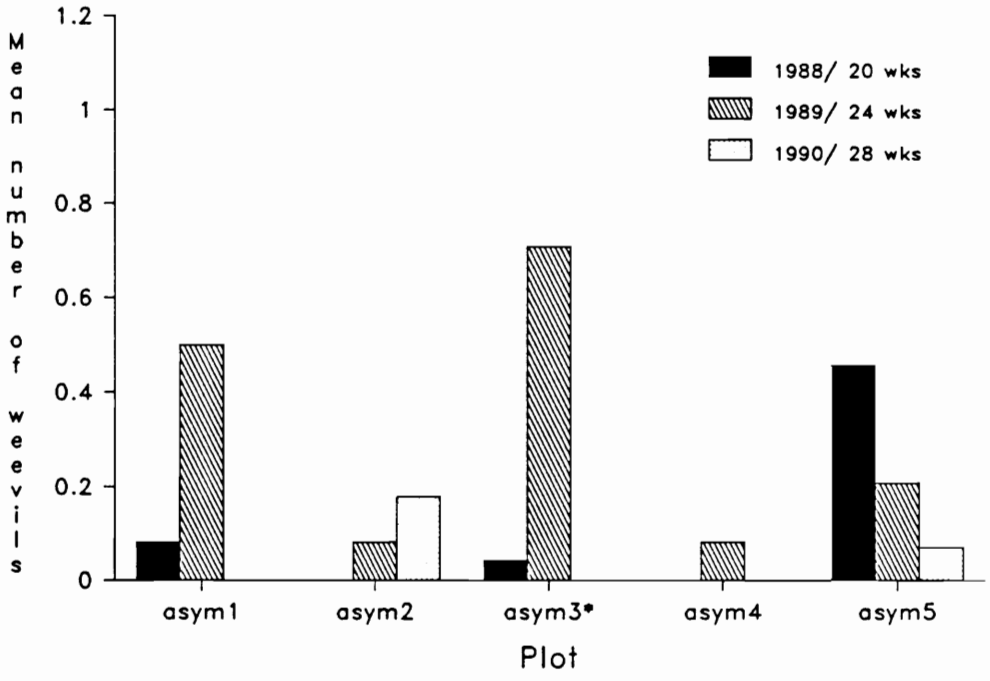

Fig. 2.3a. * PRD symptomatic trees

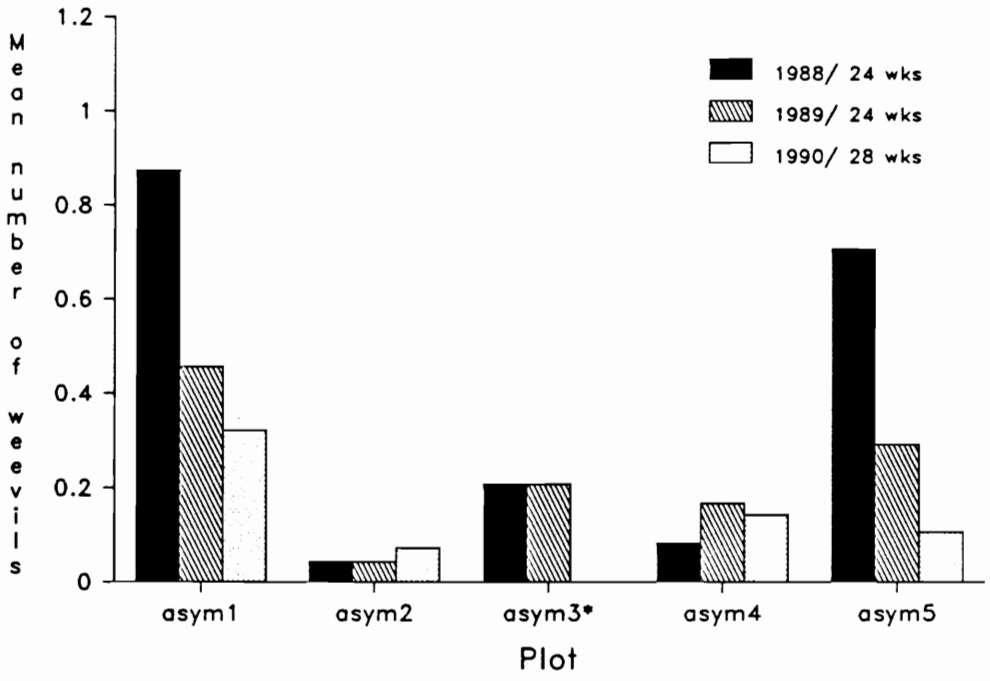

Fig. 2.3b. * PRD symptomatic trees

Fig. 2.4. Seasonal distribution of *Hylobius pales* collected in a) symptomatic, and b) asymptomatic plots PRD plantations: April - September, 1988 & 1989, and March - September, 1990.

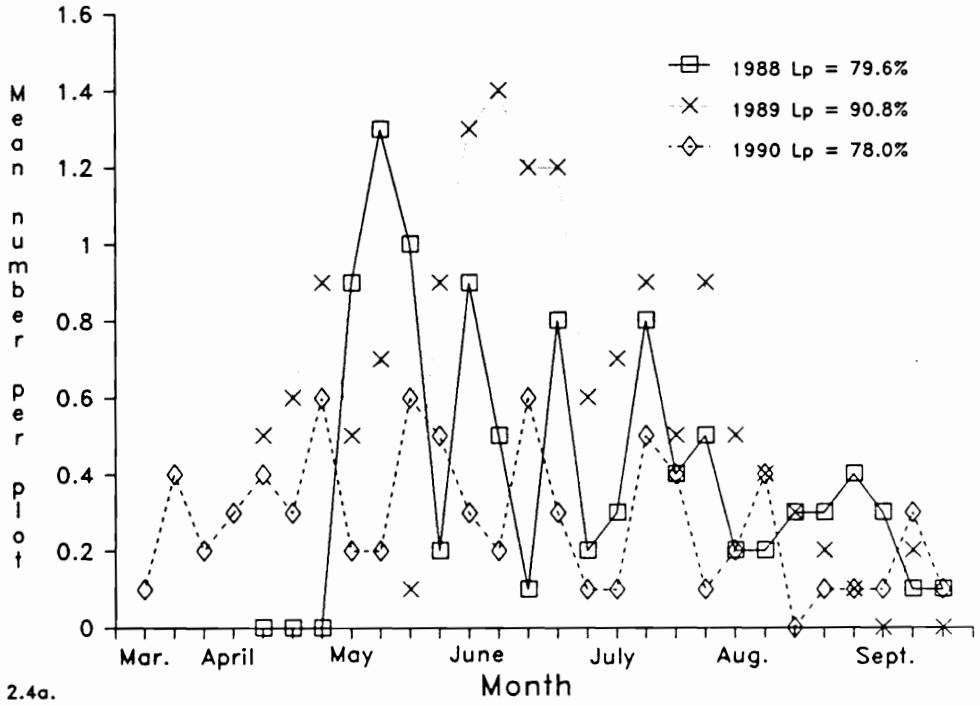

Fig. 2.4a.

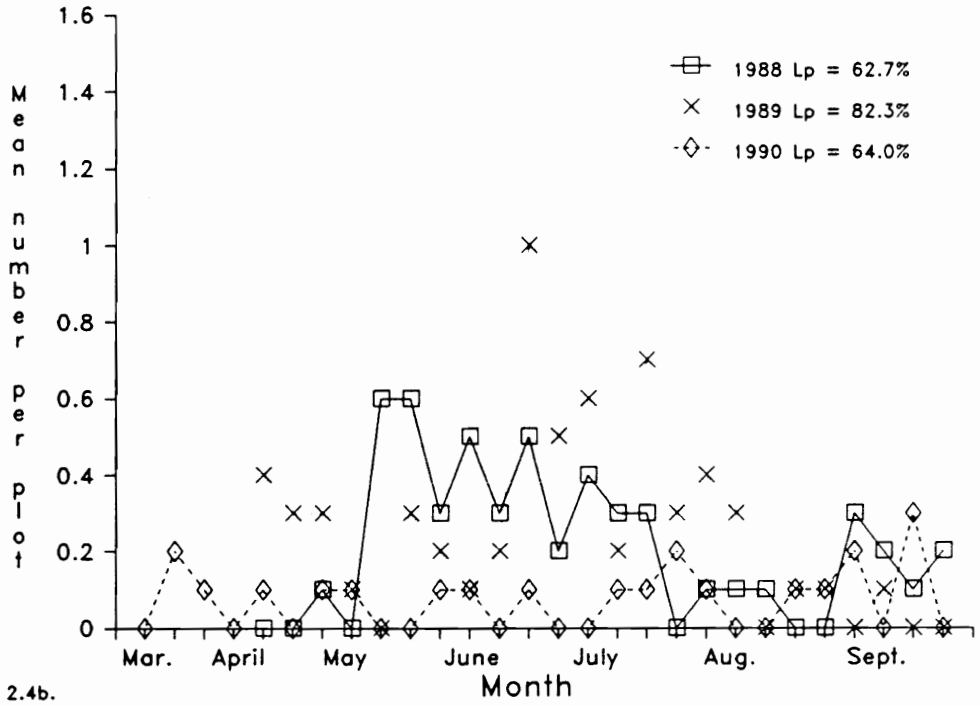

Fig. 2.4b.

Fig. 2.5. Seasonal distribution of *Pissodes nemorensis* collected in a) symptomatic and b) asymptomatic plots in PRD-symptomatic plantations: April - September, 1988 & 1989, and March - September, 1990.

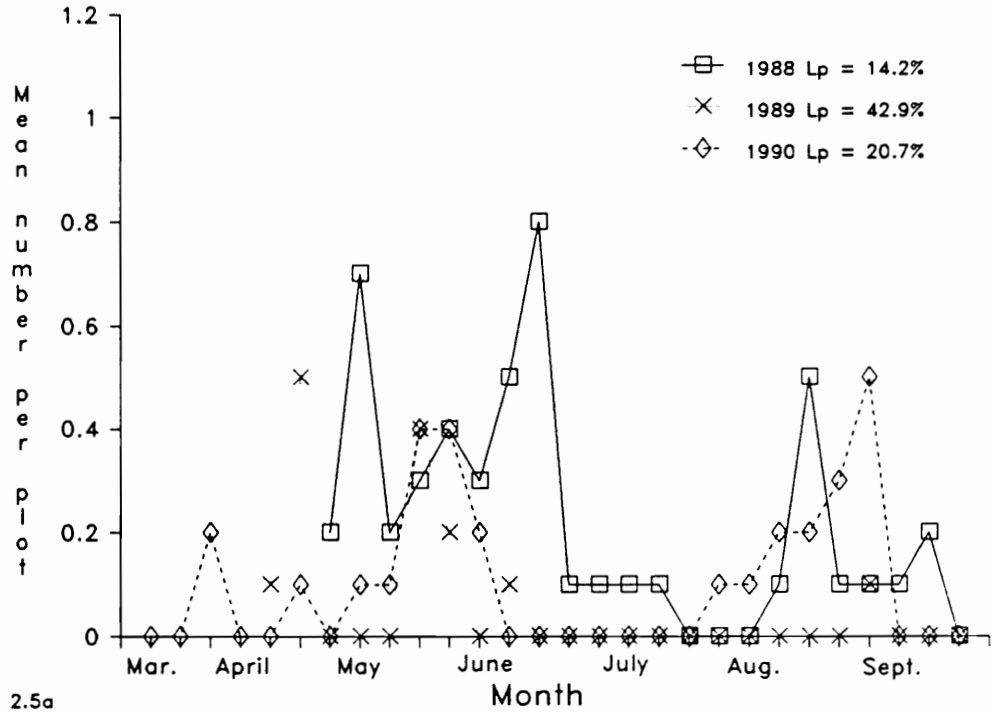

Fig. 2.5a

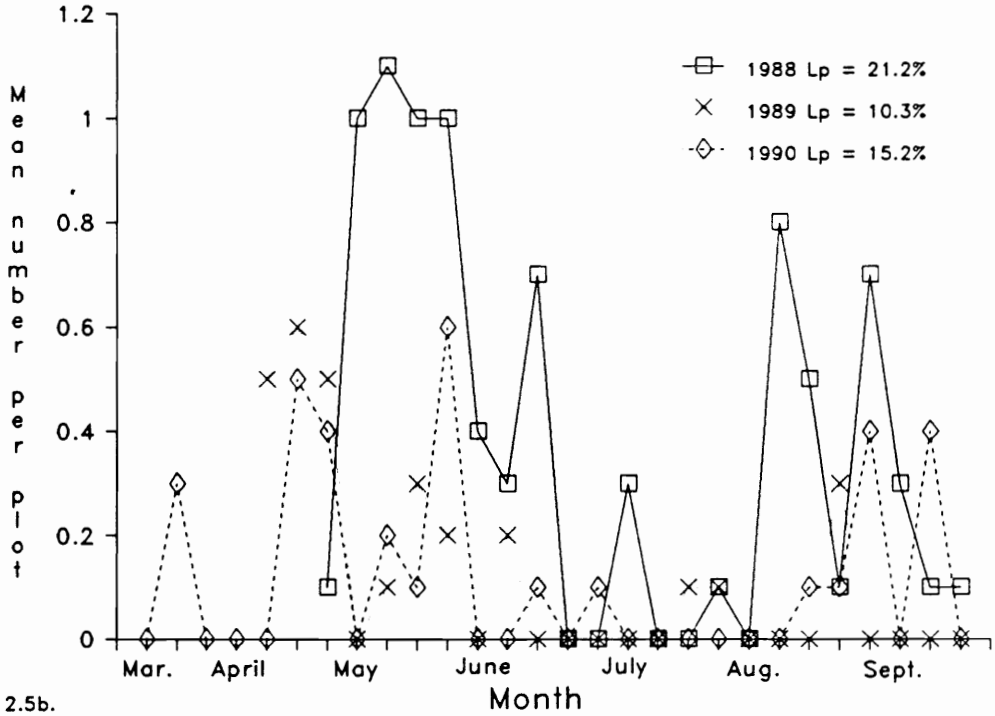

Fig. 2.5b.

Fig. 2.6. Seasonal distribution of a) *H. pales* and b) *Pissodes nemorensis* collected in PRD-asymptomatic plantations: April - September, 1988 & 1989, and March - September, 1990.

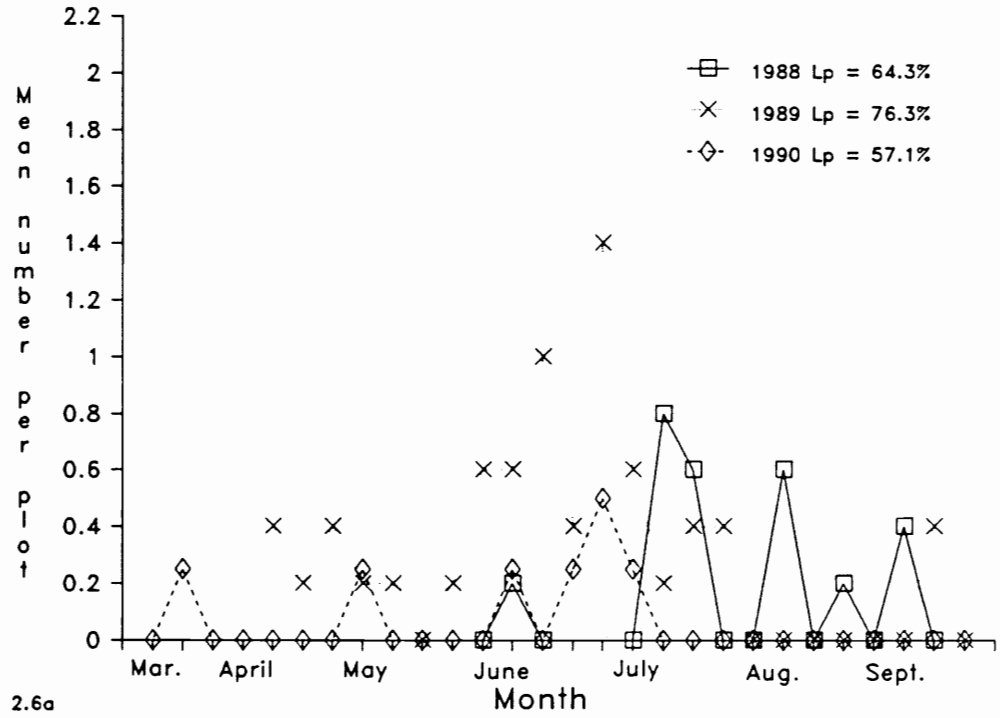

Fig. 2.6a

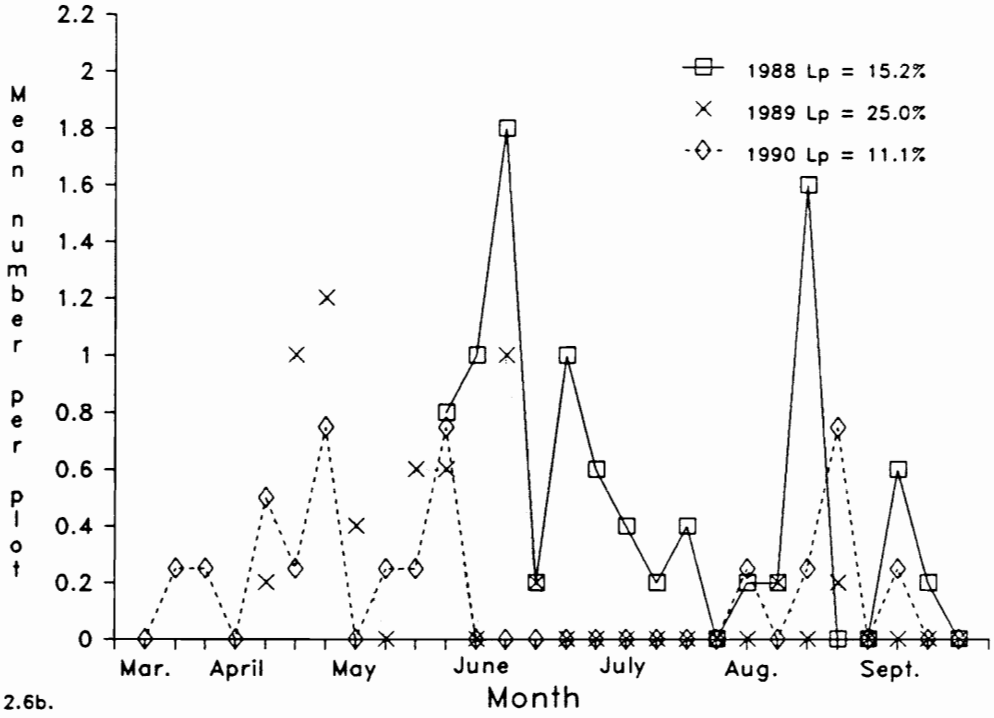

Fig. 2.6b.

Fig. 2.7. Seasonal distribution of a) *Hylobius pales* and b) *P. nemorensis* recovered in headlands 30 m from 6 Christmas tree plantations from April - September, 1990.

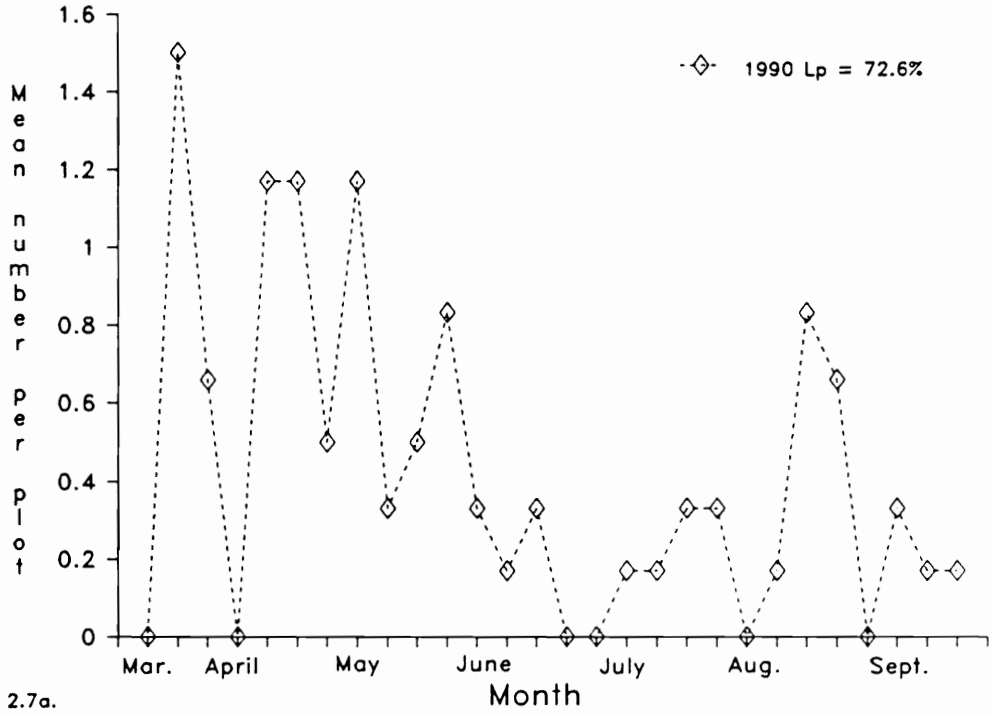

Fig. 2.7a.

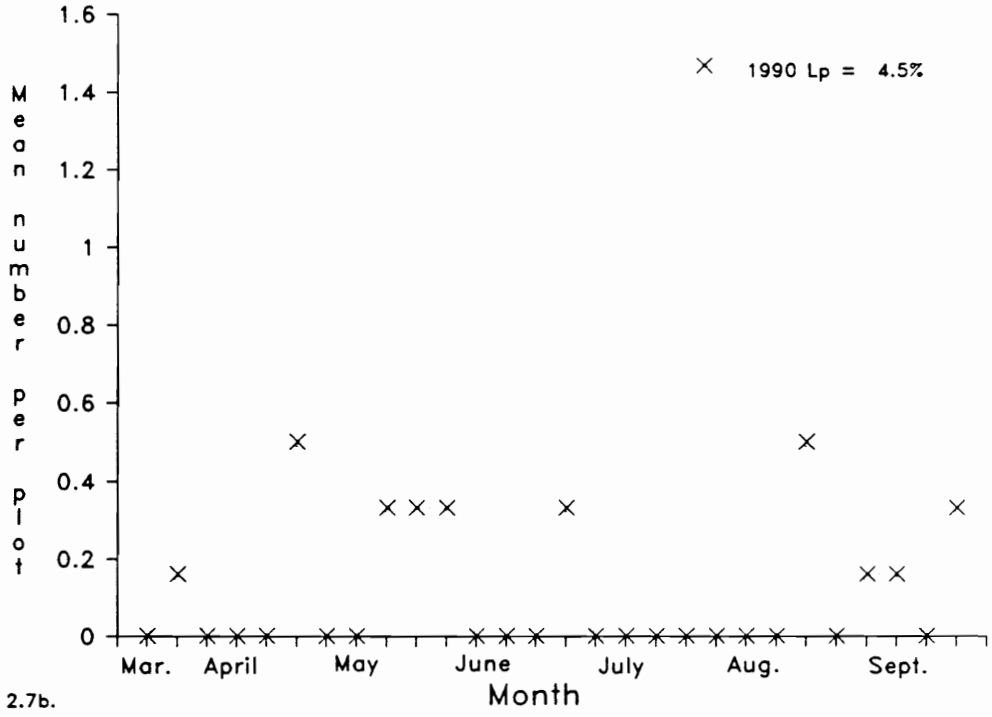

Fig. 2.7b.

Fig. 2.8. Monthly distribution of the proportion of *Hylobius pales* and *P. nemorensis* carrying *L. procerum* trapped in a) symptomatic and b) asymptomatic plots in PRD-symptomatic plantations: April - September, 1988 & 1989, and March - September, 1990.

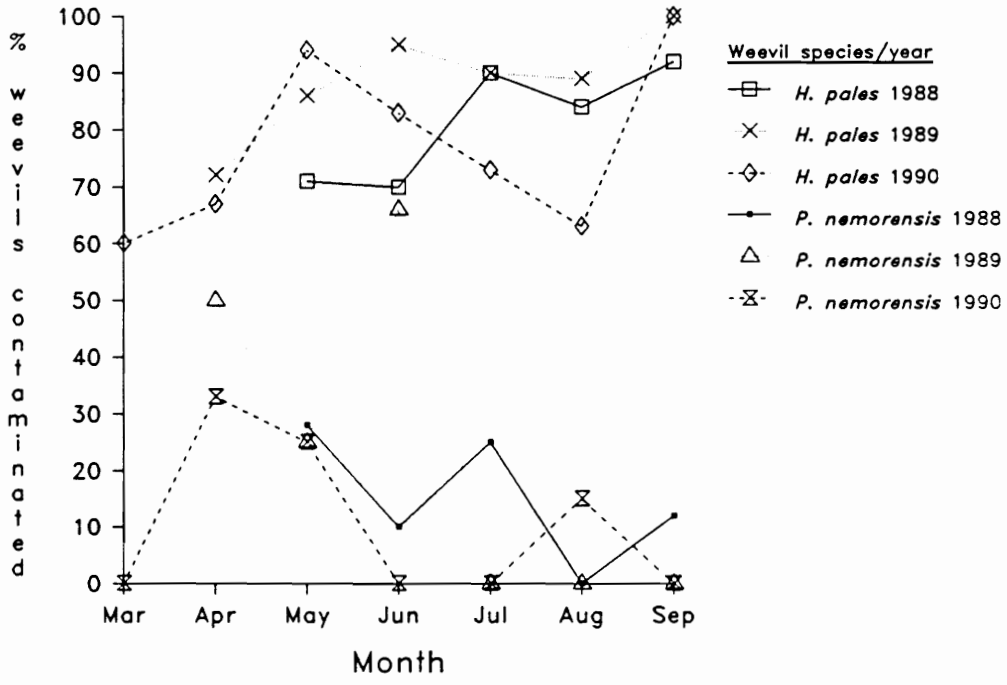

Fig. 2.8a.

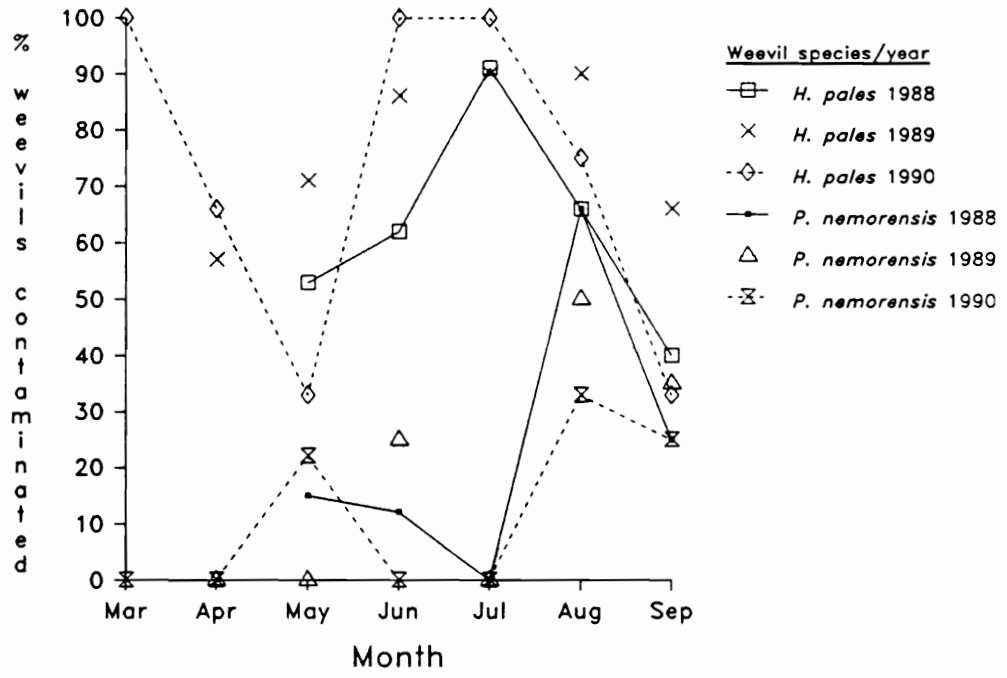

Fig. 8b.

Fig. 2.9. Progress of procerum root disease in symptomatic plots in 10 PRD-symptomatic plantations from April 1988 - September, 1990.

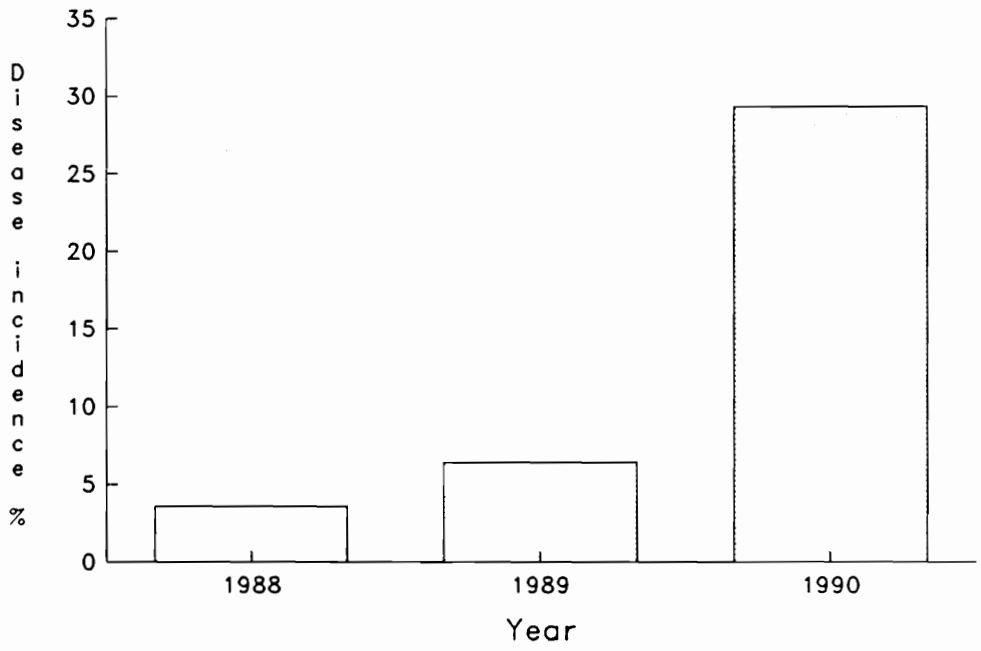

Fig. 2.9.

Chapter III

Root-Colonizing Insects and Fungi Recovered from Eastern White Pines with Procerum Root Disease

INTRODUCTION

Several authors have noted an association between procerum root disease (PRD) colonized trees with insect infestations. Lackner and Alexander (1983) recovered two weevil species, *Hylobius pales* and *Pissodes nemorensis*, their galleries, pupal chambers, and adult insects together with *Leptographium procerum* in the roots of ozone-sensitive eastern white pines along the Blue Ridge parkway in Virginia. In a second study Lackner and Alexander (1984) recovered three bark beetle genera, *Pityokteines* Fuchs, *Pityogenes* Bedel., and *Pityophthorus* Eichh. from the stems of PRD-symptomatic trees. Several of the trees were also infested with *P. nemorensis*. *L. procerum* was also isolated from trees infested by several weevil species including *H. radicus, H. pales*, *P. nemorensis*, and *Pachylobius picivorus* in Wisconsin (Wingfield 1983). Lewis and Alexander (1986) found high numbers of weevils, *H. pales* and *P. nemorensis*, carrying *L. procerum* in PRD-symptomatic and asymptomatic plantations. They also showed that the weevils were capable of transmitting *L. procerum* to uninfected pine bolts.

Although insects have been implicated as vectors of PRD, precise information detailing the host-pathogen-insect interaction has not been presented. The lack of information, tree mortality, and implication of insects as vectors of PRD have led to the present research. The objectives of this study are: 1. to describe the insect guild within roots, root collars and lower stems of healthy and infected trees; 2. to record the fungi associated with diseased trees, and determine their pathogenicity; and 3. examine apparently healthy trees in PRD-symptomatic areas of plantations for *L. procerum*.

MATERIALS AND METHODS

Symptomatic tree study. Root systems of healthy eastern white pine and those showing symptoms of PRD were excavated over a 3 year period, 1988-1990, from four Christmas tree plantations in the following locations in southwestern Virginia: Max Meadows (Wythe County), Floyd (Floyd County), Riner (Montgomery County) and Pilot (Montgomery County). At the beginning of the study, the plantations contained trees between 1-14, 3-10, 6-7 and 7-9 years old, respectively. Christmas trees had been harvested annually over 8 years at the Max Meadows plantation while harvesting at the Floyd plantation had occurred 2 years prior to the study. Harvest began at the other two plantations during the fall of the first year of the study, in 1988. The Pilot plantation contained a mixture of Christmas tree species, but the study plot was located in a 15-ha block of eastern white pine. The other plantations consisted only of eastern white pine. *Leptographium procerum* was isolated from symptomatic trees at each plantation prior to the study to confirm the presence of the disease.

In May of 1988, diseased trees from each site were assigned into categories: 1) healthy (no wilting or loss of shoot growth and no resin soaking at the root collar), 2) symptomatic green (green foliage, but visibly wilting, and resin soaking of the root collar), 3) symptomatic yellow (green-yellow needles, obvious wilting, and resin soaking of root collar), and 4) red/dead (dead trees with resin soaked collars). A fifth class was added in May of 1989: presymptomatic green, (apparently healthy green foliage, but resin soaking evident at the root collar). Healthy trees were chosen at least 10 m from visibly diseased trees at each site. One tree from each of the symptom classes at each site was excavated at monthly intervals from June to September for 1988 and 1989. In 1990, tree removal began in April and ended in

September. Excavated trees included all roots > 1.0 cm in diameter and < 20 cm from the root collar. Trees appearing long dead with peeling bark, or damaged by machinery were excluded.

At the laboratory, the roots, root collar and the lower stem was examined visually for possible feeding by insects. The bark was removed from the roots to 50 cm above the root collar. Representative subcortical insects within the lower stem and roots were collected and preserved separately and their position relative to stained wood noted. Bark beetles were identified at the Insect Identification Laboratory, VPI & SU and by Dr. D. M. Anderson, Systematic Entomology Laboratory, Plant Sciences Institute, USDA. Weevil larvae samples were identified by Dr. Tom Atkinson, Department of Entomology and Nematology, University of Florida, Gainesville, FL.

The extent of pathogen colonization of the host xylem was estimated by the amount of resin soaking present at the root collar. Sapwood samples were taken aseptically with a #4 cork borer at the roots, root collar, and 10 cm above the root collar, and plated onto actidione-malt agar (AMA) (McCall and Merrill 1980). The samples were examined after 14 days, and the fungi recovered were compared with Kendick's (Kendrick 1963) description of *L. procerum* and Upadaya's (Upadaya, 1978) description of *Ceratocystis* species. Unknown species were sent to Dr. K. Seifert, Biosystematics Laboratory, Ottawa, Ontario, Canada.

Pathogenicity study. Pathogenicity tests were conducted on the most commonly isolated identifiable species from PRD-symptomatic trees: *L. procerum*, *Ophiostoma picea* (identified by Dr. Seifert), and a *Graphium* species. Other *Ophiostoma/Ceratocystis* species, such as *O. ips,* were not identified in time to be included in this study. *O. picea* and the *Graphium* species were also contaminants of

H. pales and *P. nemorensis* (Chapter II). Both weevil species transmitted *O. picea* to seedlings (Chapter IV). Twenty eastern white pine seedlings were inoculated by removing a 5 x 10 mm piece of bark with a scalpel, inserting a similar sized block of malt extract agar (MEA) colonized by the fungus, and then wrapping with parafilm. Twenty additional seedlings were mock-inoculated with sterile MEA blocks. The trials were repeated twice, once in May and once in June, 1990.

The seedlings were held in a greenhouse for 3 months, or until disease symptoms developed. Upon examination, the stem of each seedling was surface sterilized in a 10% Chlorox solution for 10 min. The mean length of the lesion above and below the inoculation point was recorded. The bark and cambium 4 cm above and below the inoculation point were removed and plated onto AMA. The exposed stem was cut into eight 1-cm sections, four sections above and four sections from below the inoculation point. The inoculated portion was discarded and the stem sections were plated sequentially onto AMA beginning with the section adjacent to the inoculation point. After three weeks, the sections were examined for the presence of the respective fungi and the distance the fungi were recovered from above, and below the inoculation point were recorded. The mean recovery distance was calculated by combining the recovery distances of the fungi from above, and below the inoculation point. Both mean lesion length and mean recovery distance were analyzed by a one-way ANOVA using SYSTAT 4.1 (SYSTAT Inc.). Means were separated by a function using Duncan's multiple range test (SYSTAT, Inc.)

Healthy tree study. In June 1990, 20 healthy trees were excavated from the symptomatic areas of three plantations with PRD-symptomatic trees. The plantations were located at , Floyd (Floyd Co., VA), Pilot (Montgomery Co., Va),

and Riner (Montgomery Co. Va). Trees were selected on the basis of healthy green foliage and the absence of any PRD symptoms or insect activity.

At the laboratory, bark from 50 cm above the root collar and the roots was removed. Sapwood samples were taken with a #4 cork borer at the roots, root collar, 10 cm above the root collar, and plated on AMA. The samples were examined after 14 days described previously.

RESULTS

Disease symptoms. In 1989, only two presymptomatic trees, were < 50% resinous at the root collar. All the other trees of showed > 90% resinosus at the circumference of the root collar. Trees designated as healthy, including two from which *L. procerum* was isolated, showed no resin soaking at the root collar.

Fungal isolation. *Leptographium procerum* was the most commonly isolated pathogen from symptomatic trees (Tables 1) and was isolated from 38, 36 and 49% of the symptomatic trees in 1988, 1989 and 1990, respectively. Another *Leptographium* species, *L. serpens* was isolated from 6% of the trees in 1989. Other potentially pathogenic fungi included *Ophiostoma picea*, *O. ips*, *C. sagmatospora*, a *Graphium* species which produced no perfect state, and other unidentified *Ceratocystis/Ophiostoma* species. *Ophiostoma picea* was isolated from 5% of symptomatic trees in 1989 and 2% in 1990. *Ceratocystis/Ophiostoma* species were isolated from 10, 23 and 34% of symptomatic trees in 1988, 1989 and 1990, respectively. *Ophiostoma picea* was isolated together with *L. procerum* from 2% of the symptomatic trees in 1990. *Leptographium procerum* and the *Ceratocystis/Ophiostoma* species were isolated from the same tree from 2% of the symptomatic trees in 1988, 12% in 1989, and 9% in 1990. No potentially pathogenic

fungi were recovered from 48% of symptomatic trees in 1988, while in 1989 and 1990, none were recovered from 41 and 22%, respectively.

Root- and stem-inhabiting insects. Larvae of *H. pales* and *P. nemorensis* were the most consistently recovered insects from symptomatic trees. No weevil larvae were recovered from healthy trees, but they were recovered from trees in the other symptom classes, and from all of the plantations (Table 1). Collectively, weevil larvae were recovered from 52, 42, and 43% of symptomatic trees in 1988, 1989, and 1990, respectively. Bark beetle species were recovered from 29% of symptomatic trees in 1988, 20% in 1989 and 1990. Bark beetles were found in all four plantations, but were not recovered from presymptomatic trees or healthy trees. Genera of bark beetles (family Scolytidae) recovered included: *Pityogenes* Bedel, *Xylerborus* Eich., *Orthotomicus* Eich., *Ips* Degeer and *Pityophthorus* Eichh. One member of the Colydiidae family was also recovered. Bark beetles identified to species included *Pityogenes hopkinsi* Swaine, *Ips pini* (Say), and *Orthotomicus caelatus* Eich.

Weevils and bark beetles were recovered from 58% of the symptomatic trees in 1988, 47% in 1989, and 51% in 1990 while healthy trees were free from insects and insect damage. Insects were most frequently recovered from the stem or root collar region. Weevil larvae were recovered mostly from the lower stem and root collar area, and were recovered from the roots of only 1% of the trees during the study. In one instance, weevil larvae were recovered one meter above the root collar. Bark beetles were not found in the roots and were generally located in the stem above resin soaked tissue and were rarely found in resin soaked tissue near the root collar.

Weevil larvae and *L. procerum* were recovered together from 23% of the symptomatic trees in 1988, 17% in 1989, and 21% in 1990. Conidiophores of *L. procerum* were found sporulating in weevil galleries in two symptomatic trees in 1989 and one in 1990. A total of 22 *P. nemorensis* and two *H. pales* preemergent adults were recovered from 8 symptomatic trees, with five *P. nemorensis* and two *H. pales* contaminated with *L. procerum*. Four weevil larvae were also found to be contaminated with the fungus.

Possible weevil feeding was observed on 5% of the symptomatic trees in 1988, 4% in 1989 and 7% in 1990. However, the apparent feeding may have been oviposition chambers, as weevil larvae were recovered from all but one of the trees where feeding wounds occurred. Feeding wounds of *H. pales* were observed at the root crotches of one healthy tree from which *L. procerum* was recovered in 1990.

Pathogenicity study. The mean lesion length and the mean recovery distance of the fungus in the sapwood was significantly greater ($P < 0.05$) for the seedlings inoculated with *L. procerum* than those of seedlings inoculated with *O. picea* and the *Graphium* spp (Table 2). The mean lesion length and the mean recovery distance of *O. picea* in the sapwood was intermediate between *L. procerum* and the *Graphium* spp.

The mean lesion length in seedlings inoculated with *L. procerum* was 2 x that of seedlings inoculated with *O. picea* and the *Graphium* spp. (Table 2). In the first trial, *L. procerum* was recovered from the sapwood, on average, a distance 6 x > the mean lesion length. Both *L. procerum* and *O. picea* were isolated up to 4 cm from the point of inoculation although there was no dark staining in the sapwood beyond the extent of the lesion. In both trials, all seedlings inoculated with *L. procerum* were resinous at the end of the study period. In the first trial 25% of the seedlings

inoculated with the *Graphium* spp. and 15% of the *O. picea*-inoculated seedlings were not resinous and showed no lesion growth. In the second trial 15% of the *Graphium* spp. inoculated seedlings and 20% of the *O. picea*-inoculated seedlings had no lesion extension and were not resinous. Only 10% of the *L. procerum*-inoculated seedlings showed no lesion growth.

At the end of the study period, one control, one *L. procerum*-, and one *O. picea*-inoculated seedling died from the first trial, and one *L. procerum*-inoculated seedling from the second trial. Fungi recovered from the mock-inoculated seedlings included zygomycetes and species of *Penicillium*, but no identifiable pathogenic fungi. The bark and sapwood from the *L. procerum*- and *O. picea*-inoculated seedlings were resin soaked 1 cm above and below the vicinity of the inoculation point.

Healthy tree study. *Leptographium procerum* was recovered from 18% of the apparently healthy trees excavated from symptomatic areas of three PRD symptomatic plantations (Table 3). *Ophiostoma picea* was recovered from 5% of the trees at one site and *Ceratocystis* species were recovered from 10% the trees at two sites. Feeding wounds by *H. pales* were observed on 20% of the trees from the three plantations and *L. procerum* and feeding wounds occurred together on 5% of the trees. Insect feeding was not detected on the other trees from which *L. procerum* was recovered. Between 5 - 10% of the trees had resin soaked areas in the sapwood, but no pathogenic fungi were recovered.

DISCUSSION

The hypothesis that insects are the vectors of *L. procerum* was first set forth by Wingfield 1983, who isolated the fungus from insect damaged trees and from the adults of insects which infest the roots, and root collars of pines, primarily

Dendroctonus valens, Hylobius pales, H. rhizophagus and *Pachylobius picivorus*. Lackner and Alexander (1984) extended the list to include *Pissodes nemorensis*. This study confirms and extends the insect-pathogen association for *H. pales* and *P. nemorensis* by demonstrating that these insects consistently colonized diseased eastern white pine in all stages of decline.

It is doubtful if the insect-pathogen association also extends to *Ips* species collected. Although they were collected from colonized tissues, *Ips* species usually do not attack healthy trees (Drooz 1985) and in this study they were recovered only from trees in the later stages of decline.

The results of this study provide evidence for the first and second of the four postulates proposed by Leach (1940) for identifying insect vectors of plant diseases: 1) a strong association of insects and diseased plants (Table 1), 2) demonstration that insects regularly visit healthy plants under conditions suitable for the transmission of the disease (Table 3). The pattern of insect colonization of infected root collars follows the pattern of colonization by *L. procerum*: as the pathogen spreads throughout the root collar, the infected tissue becomes suitable material for oviposition and brood development. During gallery and pupal chamber construction the insects become contaminated by the fungus as demonstrated by the recovery of *L. procerum* from weevil larvae and preemergent adults. There is little evidence to support the possibility that *L. procerum* was introduced during oviposition as the fungus was isolated from PRD-symptomatic trees not colonized by insects. The fungus was isolated, however, from trees where feeding wounds of *H. pales* had occurred. Feeding wounds and the recovery of *L. procerum* occurred together on both PRD-symptomatic trees and apparently healthy trees.

Leptographium procerum was recovered from the sapwood, on average, a distance 4.5 x greater than the average lesion length. The sapwood of these seedlings was not stained beyond the lesion. A mechanism for this observation may be provided by Bertagnole *et al.* (1982) who showed that the yeast-like form of *L. procerum* could be transported in the xylem without producing demitiaceous hyphae. *Ophiostoma picea* was also recovered well in advance of the lesion and may be transported in the sapwood by a similar mechanism.

Leptographium procerum was the most pathogenic of the fungi recovered from symptomatic trees. The mean lesion length of *L. procerum*-inoculated seedlings was twice that of *O. picea*- and the *Graphium* spp. inoculated seedlings. However, both species produced lesions significantly greater than the control and may be considered weak pathogens compared to *L. procerum*.

Pathogenicity tests of other *Ceratocystis/Ophiostoma* species recovered from symptomatic trees, such as *O. ips*, and *C. sagmatospora* have not been conducted on eastern white pine. Raffa (1988), found inoculated *Pinus banksiana* and *P. resinosa* to contain *O. ips* by forming necrotic lesions, however, similar observations were made when the same pine species were inoculated with *L. procerum* (K. Raffa personal communication). The pathogenicity tests conducted in this study indicate that *O. picea* and the *Graphium* spp. are weak pathogens and that *L. procerum* is the causal agent of PRD. Additional pathogenicity tests of the other *Ceratocystis/Ophiostoma* species, such as *O. ips,* recovered from PRD-symptomatic trees would remove any further doubt.

L. procerum was recovered from 18% of apparently healthy trees in PRD-symptomatic areas of three plantations. These findings indicate that in addition to

visual symptoms, such as discolored foliage and resin soaking at the root collar, another measure of infection and colonization is needed.

Table 3.1. Percentage of occurrence of insects, mean numbers of weevil larvae per tree, the percentage occurence of L. procerum and of insects, and pathogenic fungi recovered from healthy and procerum root disease symptomatic eastern white pines 1988 - 1990.

	Percentage of trees								
Symptom class	Weevil larvae	Weevil larvae and L. procerum	Bark beetles	Bark beetles and L. procerum	L. procerum	L. serpens	Ophiostoma piceae	Ophiostoma/ Ceratocystis spp.	Ophiostoma/ Ceratocystis spp. and L. procerum
1988[1]									
Green	0 (0)[3]	0	0	0	0	0	0	0	0
Presymptomatic green	- (-)	-	-	-	-	-	-	-	-
Symptomatic green	37.5 (4.0)	25.0	12.5	6.3	37.5	0	0	6.3	0
Symptomatic yellow	56.3 (2.6)	25.0	43.8	18.8	50.0	0	0	18.8	6.3
Symptomatic red	62.5 (3.4)	18.8	31.3	6.3	25.0	0	0	6.3	0
1989[1]									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	6.3 (0.1)	0	0	0	31.3	6.3	6.3	6.3	0
Symptomatic green	43.8 (6.4)	18.8	12.5	12.5	37.5	0	0	31.3	18.8
Symptomatic yellow	68.8 (6.4)	31.3	37.5	18.8	37.5	0	6.3	25.0	18.8
Symptomatic red	50.0 (2.6)	18.8	31.3	12.5	43.8	12.5	6.3	37.5	12.5
1990[2]									
Green	0 (0)	0	0	0	8.3	0	0	12.5	4.2
Presymptomatic green	12.5 (0.8)	0	0	0	66.7	0	8.3	25.0	16.7
Symptomatic green	37.5 (4.3)	16.7	37.5	12.5	50.0	4.2	0	20.8	8.3
Symptomatic yellow	66.7 (9.3)	41.7	20.8	8.3	45.8	0	0	29.2	4.2
Symptomatic red	54.2 (6.3)	25.0	20.8	12.5	33.3	0	0	54.2	12.5

[1] n = 16 trees/class.

[2] n = 24 trees/class.

[3] indicates mean number of weevil larvae recovered per tree.

Table 3.2. Pathogenicity of fungi recovered from procerum root disease symptomatic trees determined by lesion length and distance in sapwood fungi were isolated from inoculation point, after 3 mo., in 20, 3-yr-old eastern white pine seedlings.

Inoculation	Trial 1		Trial 2	
	Mean lesion length (mm)	Mean distance in sapwood (mm)	Mean lesion length (mm)	Mean distance sapwood (mm)
Leptographium procerum	5.00[a1]	32.50[a]	6.83[a]	19.00[a]
Ophiostoma picea	2.20[b]	25.25[b]	2.25[b]	16.75[a]
Graphium spp.	1.15[c]	5.25[c]	2.10[b]	6.75[b]
Control	0.50[c]	0.00[d]	0.00[c]	0.00[c]

[1] numbers followed by the same superscripts are not significantly different at P = 0.05, using Duncan's multiple range test.

Table 3.3. The number trees with evidence of weevil feeding, or from which pathogenic fungi were recovered from 20 apparently healthy eastern white pines in symptomatic areas of 3 procerum root disease symptomatic plantations.

	No. of trees with insect feeding and fungi				
Plot	Weevil feeding	Leptographium procerum	Ophiostoma picea	Ceratocystis ips	Other Ophiostoma/ Ceratocystis spp.
PRD1	4	4 (1)[1]	1	1	1
PRD5	6	6 (1)	0	1 (1)	1
PRD11	3	1 (1)	0	0	0

[1] numbers in parentheses indicate number of trees weevil feeding and pathogen were recovered together.

Chapter IV

Transmission of *Leptographium procerum* to Eastern White Pine by *Hylobius pales* and *Pissodes nemorensis*

INTRODUCTION

Hylobius pales and *Pissodes nemorensis* have been implicated as vectors of PRD in southwestern Virginia (Lackner and Alexander 1984; Lewis and Alexander 1986). Both species are capable of feeding on stems and roots of eastern white pine (Drooz 1985; Raffa personal communication), and feeding by *H. pales* has been found during routine inspection of Christmas tree plantations (Chapter II). Lewis and Alexander (1986) found 64% of weevils recovered in a four week study to be contaminated with *Leptographium procerum*. They also showed that *H. pales* are capable of transmitting *L. procerum* to fresh eastern white pine bolts. Between 70 - 85% of the *H. pales* and 10 - 20% of the *P. nemorensis* populations described in Chapter II were contaminated with *L. procerum*. The insects may become infested with the fungus during brood development in trees killed by *L. procerum*, or by inoculation of the fungus into healthy stumps during oviposition as suggested by Witcosky *et al.* (1986). However, despite the association of weevils with procerum root disease no study has demonstrated the ability of the weevil to transmit the fungus to living trees.

The objectives of this study were to investigate the ability of weevils to transmit *L. procerum* to eastern white pine seedlings and 5-year-old trees, and to determine if oviposition in bolts by *L. procerum* contaminated adults leads to contamination of the brood by the fungus. Transmission of the fungus to seedlings by insects would complete the fourth of Leach's postulates: insects can transmit the disease to living plants.

MATERIALS AND METHODS

Seedling transmission study. *Hylobius pales* and *P. nemorensis* adults were collected from Christmas tree plantations described in Chapter II. To establish the ability of *H. pales* to transmit *L. procerum* during feeding, 20 weevils artificially contaminated with *L. procerum,* and 20 field collected weevils were individually caged on 2-year-old eastern white pine seedlings for 24 hours. The weevils were artificially contaminated by allowing them to walk across a petri plate containing *L. procerum* conidiophores growing on malt-extract agar (MEA). Cages consisted of 10 oz Styrofoam cups with lids. The cups and lids were slit on one side to allow ease of assembly with a hole in the center to accommodate the seedling's stem. Modelling clay was placed at the bottom and lid of the cup around the stem, and masking tape was placed along the slit side of the cup to keep the weevil from escaping through cracks.

To establish a standard in order to evaluate the efficiency of the insects to transmit the fungus, 20 eastern white pine seedlings were inoculated with *L. procerum* by removing a 5 x 10 mm piece of bark with a scalpel and inserting a similar sized block of malt-extract agar (MEA) colonized by the fungus, then wrapped with parafilm. Twenty additional seedlings were mock-inoculated with sterile MEA blocks. The trials were repeated three times in 1989, twice in July and once in September.

The seedlings were held in a greenhouse for 6 months, or until disease symptoms developed. Upon examination, each seedling was surface sterilized in a 10% Chlorox solution for 10 min. The bark, including the cambium, from the area of the stems fed on by the weevils was removed and plated onto actidione malt agar (AMA) (McCall and Merrill 1980). The remaining portion of the stem was cut into

1-cm sections and plated separately on AMA. Inoculated and mock-inoculated seedlings were also debarked and the bark plated on AMA. Total lesion length was measured and four 1-cm sections from above and below the inoculation point were plated sequentially from the inoculation point onto AMA. After 3 weeks, the isolates were examined for conidiophores of *L. procerum.*

The study was repeated in 1990 and the following changes made: the number of seedlings in each treatment was reduced to ten, the sterile MEA plate used for the mock inoculations was kept for 3 weeks and examined for possible contamination by *L. procerum*, and seedlings were held in the greenhouse only 3 months before examination. Trials using *H. pales* were repeated five times: three times in May and twice in June 1990. In addition, three trials were conducted using *P. nemorensis*: twice in May and once in June 1990. For trials with *P. nemorensis*, only seven seedlings were used per treatment.

Transmission to five-year-old trees. To confirm the ability of *H. pales* to transmit *L. procerum* to older trees, *H. pales* were artificially contaminated with the fungus and caged on six five-year-old eastern white pines. The trees were located in a 0.25-ha Christmas tree-like setting on the Virginia Tech campus. The trees were spaced 1.8 m apart, with 1. 8 m between trees in a given row. The weevils were contaminated with the fungus by the method described above and two weevils were caged per tree for five days. Cages consisted of 10 x 10 x 8 cm, 8 oz. plastic containers with the lids and sides of the container slit along one side to allow ease of assembly, and a hole in the center to accommodate the stem. To keep the weevils from escaping through cracks, modelling clay was placed at the bottom of the container and on the lid around the stem, and masking tape was placed along the slit side and lid.

To establish a standard in order to evaluate the efficiency of the weevils to transmit *L. procerum*, four five-year-old trees were inoculated with the fungus by removing a bark plug from the four cardinal directions around the base of each tree with a # 5 cork borer, approximately 10 cm above the ground. An equivalent sized plug of MEA colonized by the fungus was inserted into the holes and wrapped with parafilm. Four other trees were mock-inoculated using sterile MEA plugs, and another four trees were retained as untreated controls.

The trees were observed for 5 months before examination. At the time of evaluation, each tree was first surface sterilized with 70% EtOH. The bark, including the cambium, from the area of the stems fed on by the weevils was removed and plated onto AMA. Preference was given to areas of the bark where weevil feeding had occurred. Sapwood samples were taken from the remaining portions of the stem with a #5 cork borer with preference given to areas with evidence of weevil feeding. Inoculated and mock-inoculated trees were also debarked and the bark plated onto AMA. Total lesion length and isolations were taken from two of the four inoculation points made at the base of the trees. Four 1-cm samples from above and below the inoculation point were taken with a #5 cork borer and plated sequentially from the inoculation point onto AMA. Untreated control trees were debarked, and bark and sapwood samples were taken randomly from the area 10 - 15 cm above the root collar. After 3 weeks, the isolates were examined for conidiophores of *L. procerum.*

Colony development. A modification of the procedure proposed by Speers and Cody (1975) was used to rear *H. pales*. For oviposition, three split billets 20 cm long and 5 - 8 cm in diameter were placed in a clear plastic box, 20 x 40 x 10 cm, together with field collected *H. pales*. The weevils were allowed to feed and oviposit

on the billets for one week. The billets were removed to another clear plastic box, 20 x 40 x 10 cm, with moistened paper towels on the bottom and maintained at room temperature. The sets of billets were replaced every week for 4 weeks. The billets were examined after 4 weeks for *L. procerum* by culturing pieces of wood and bark, or by observing conidiophores of the fungus growing directly on the billets.

After emergence, contamination by *L. procerum* was checked by placing the weevils on AMA plates for 24 hours. They were then removed to a separate plastic box and given fresh billets weekly for 3 weeks. The billets were placed in a plastic box, 20 x 40 x 10 cm, with moistened paper towels and maintained at room temperature. After 4 weeks, transmission of *L. procerum* was determined by observing conidiophores the fungus growing directly on the billets. Upon emergence of the second generation, the weevils were placed on AMA to determine whether they were contaminated with *L. procerum*.

RESULTS

1989 weevil transmission studies. None of the seedlings were girdled by weevil feeding and the first seedling died 30 days after feeding had occurred. Feeding by both artificially contaminated and field collected *H. pales* resulted in the transmission of *L. procerum* to the eastern white pine seedlings (Table 1). The artificially infested weevils were able to transmit the fungus to 100% of the seedlings in trial 1, 90% in trial 2, and 85% in trial 3 (Table 1). The field collected weevils transmitted *L. procerum* to 55%, 80% and 35% of the seedlings in trials 1, 2, and 3, respectively. In addition to *L. procerum*, field collected weevils also transmitted *Ophiostoma picea* to 15% of the seedlings in the second trial and 75% in the third. In the third trial *O. picea* was also recovered from 35% of seedlings from which *L. procerum* was not found. By the end of the study period, 18% of the seedlings fed

on by the field collected weevils and 13% of those fed on by artificially infested weevils had died.

Hylobius pales fed on the seedlings by consuming the bark and cambium to the sapwood. Feeding was spotty, but sometimes would continue for one to two centimeters up the stem. Some wounds were not completely callused by the end of the test period and several were resinous. *Leptographium procerum* was recovered from all feeding wounds that were not completely callused or that produced resin.

Wounds created to inoculate seedlings with agar colonized by *L. procerum* varied from being completely callused, to callus production only at the lateral edges of the wound and a lesion extending from 0.2 to > 2cm up and down the stem. Five percent of the *L. procerum* inoculated seedlings died before the end of the 6 month incubation period.

Wounds of the mock-inoculated seedlings were completely callused, but *L. procerum* was recovered from between 15 - 50% of the plants. *Leptographium procerum* was recovered from the region adjacent the inoculation point suggesting that an error in technique had occurred and the agar used for mock-inoculation had become contaminated by the fungus. *Leptographium procerum* was isolated from one of two mock- inoculated seedling that died before the end of the incubation period.

1990 Weevil transmission studies. In 1990, feeding by both weevil species resulted in the transmission of *L. procerum* to the eastern white pine seedlings (Tables 1, 2). Feeding by *H. pales* and *P. nemorensis* artificially contaminated with *L. procerum* transmitted the fungus to 90 - 100% and 100% of the seedlings, respectively. Field collected *H. pales* transmitted *L. procerum* to 50 - 80% of the seedlings while field collected *P. nemorensis* did so to only 15 - 43%. *Ophiostoma*

picea was transmitted to 10 - 60% of the seedlings fed on by field collected *H. pales* and to 30% by field collected *P. nemorensis*. During the study one seedling fed on by artificially infested *P. nemorensis* died.

Feeding wounds created by *H. pales* were similar to those observed in 1989. Wounds created by *P. nemorensis* feeding were smaller than those of *H. pales*. *P. nemorensis* feed by chewing a small hole in the bark, inserting their beaks, and consuming the cambium under the bark. Feeding was always spotty around the seedling stem. After three months, many of the wounds made by *P. nemorensis* feeding were resinous and *L. procerum* was recovered from these wounds.

Wounds of seedlings inoculated with agar colonized by *L. procerum* varied from being completely callused, to a lesion extending from 0.2 to > 2cm up and down the stem. Only three of the seedlings inoculated with *L. procerum* died during the three month incubation period.

Leptographium procerum was not recovered from any of the mock-inoculated seedlings in 1990, all wounds were completely callused and none died by the end of the study. *Leptographium procerum* was not recovered from any of the MEA plates used for mock-inoculation.

Five-year-old tree transmission study. The weevils fed on all of the saplings and *L. procerum* was recovered from 100% of the trees. Feeding wounds occurred as ragged irregular pits to the sapwood and were similar to those found on mature trees in Christmas tree plantations described in Chapter II. Although after 5 months some of the feeding wounds were resinous and *L. procerum* was recovered from these areas, none of the trees in this study showed foliar symptoms of disease at the time they were harvested.

Wounds created to inoculate the trees with *L. procerum* colonized agar were callused, but all wounds had resin actively flowing at the end of five months. The fungus was recovered up to 3 cm from the inoculation point although there was no discoloration of the sapwood

Wounds of the mock-inoculated trees were completely callused although three of the wounds were still resinous at the end of the study. *Leptographium procerum* was not recovered from any of the mock-inoculated trees nor was it recovered from any of the untreated controls.

Hylobius pales **colony development.** Twenty-three weevils emerged over a four week period from the billets oviposited on by field collected *H. pales*. The first weevils were observed 55 days after the first billets were removed. One hundred-percent of the weevils were contaminated with *L. procerum* and the fungus was observed sporulating on all but one of the 12 billets. Conidia were observed sporulating at feeding sites, in larval galleries, and in pupal chambers. Bark and wood cultures from the remaining billet yielded *L. procerum*.

Only one weevil emerged from the billets oviposited on by the first generation of the colony, however, the one emerging weevil was contaminated with *L. procerum* and *O. picea.*

CONCLUSIONS

Field collected weevils of both species and those artificially infested with spores of *L. procerum* were able to transmit the fungus to eastern white pine seedlings. Artificially contaminated *H. pales* were also able to transmit the fungus to five-year-old trees. The ability of the artificially contaminated *H. pales* to feed on the rough bark of five-year-old trees confirms field observations, documented in Chapters II and III, that the weevils feed at the base of mature Christmas trees.

Field collected *P. nemorensis* transmitted *L. procerum* to fewer seedlings than did field collected *H. pales*. However, this was expected as *P. nemorensis* are contaminated less frequently with the fungus (Chapter II). Transmission of the fungus by both species resulted in disease development. In 1989, 2 x as many seedlings fed on by both field collected and artificially inoculated *H. pales* died than seedlings inoculated with agar colonized by *L. procerum*. These results show that *H. pales and P. nemorensis* carry sufficient *L. procerum* inoculum to transmit the fungus to seedlings and cause disease. These results also fulfill the fourth of Leach's postulates of proof of insect transmission of a plant disease: insects successfully transmit the pathogen to plants under controlled conditions (Tables 1 - 2).

Another fungus, *O. picea*, was also transmitted to seedlings by feeding by both weevil species. Transmission of this fungus occurred both separately and in conjunction with *L. procerum*. From the results in Chapter III, *O. picea* may be moderately pathogenic to eastern white pine. This is the first record of the transmission of this fungus by insects to eastern white pine.

Field collected weevils carried sufficient *L. procerum* to inoculate breeding material with the result that the emerging brood was also contaminated with the fungus. Moreover, the emerging brood also carried sufficient inoculum to inoculate new breeding material and contaminate a second brood. Thus two generations of weevils raised under controlled conditions from field collected weevils were contaminated with *L. procerum*. These results demonstrate that *H. pales* infested with *L. procerum* may inoculate uninfected breeding material, such as stumps, and that the emerging brood may be contaminated with the fungus.

Table 4.1. The number of seedlings from which Leptographium procerum was recovered after feeding by artificially infested or field collected H. pales, and inoculation with L. procerum colonized agar or sterile agar in 1989.

Year/ trial	Treatment			
	Artificially infested weevils	Field collected weevils	L. procerum colonized agar	Sterile agar
1989[1]				
1	20^{a4}	11^{b}	20^{a}	3^{c}
2	17^{a3}	16^{b3}	20^{a}	10^{c}
3	17^{a}	7^{b}	20^{a}	6^{b}
1990[2]				
1	10^{a}	5^{b}	10^{a}	0^{c}
2	10^{ab}	8^{b}	10^{a}	0^{c}
3	9^{a}	8^{b}	10^{a}	0^{c}
4	10^{a}	7^{b}	10^{a}	0^{c}
5	10^{a}	6^{b}	10^{a}	0^{c}

[1] 20 seedlings per treatment unless noted.

[2] 10 seedlings per treatment.

[3] 19 seedlings per treatment

[4] values followed by the same letter, within a row, are not significantly different (p = 0.05 Chi-squared test)

Table 4.2. The number of seedlings from which Leptographium procerum was recovered after feeding by artificially infested or field collected P. nemorensis, or inoculation with L. procerum colonized agar or sterile agar in 1990.

Trial	Treatment			
	Artificially infested weevils	Field collected weevils	L. procerum colonized agar	Sterile agar
1	7^{a1}	1^{b}	7^{a}	0^{b}
2	7^{a}	3^{b}	7^{a}	0^{c}
3	7^{a}	2^{b}	7^{a}	0^{c}

[1] values followed by the same letter are not significantly different (p = 0.05 Chi-squared test)

* 7 seedlings per treatment unless noted.

** 7 seedlings per treatment

Chapter V

SUMMARY

The results of this study fulfill all of Leach's Laws for identifying insect vectors of plant disease: a) both *H. pales* and *P. nemorensis* are constantly associated with diseased trees as shown by the recovery of their larvae from trees in various stages of decline caused by PRD; b) both visit healthy trees under conditions suitable for transmission as noted by feeding on the stems and roots of apparently healthy trees and the recovery of *L. procerum* from these sites; c) both carry inoculum in the field, from collections in traps in Christmas tree plantations over three field seasons; and d) field collected weevils of both species transmit the disease under laboratory conditions to seedling eastern white pine.

Thus an intimate association exists between the fungal pathogen, *L. procerum*, the insect vectors, *H. pales* and *P. nemorensis*, and their mutual host, eastern white pine. The larvae of the insect vectors often develop in host brood trees killed by the fungus. During their development the insects become contaminated with the fungus and carry it on their body parts when they emerge. Contaminated insects transmit the fungus during normal feeding activity on the stems of seedlings and older trees. After incubation of the fungal pathogen and expression of disease, the insect may vectors revisit the killed host to brood and the cycle is repeated.

Of the two vector species, *H. pales* was recovered and was contaminated with *L. procerum* more frequently than *P. nemorensis*. Which species is the most effective vector in the field was not determined conclusively in this study, but *H. pales* is favored because of the high numbers carrying the fungus and the ability of field collected weevils to transmit *L. procerum*.

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APPENDIX

APPENDIX A

Evaluation of Annual Lindane Applications to Control Weevils and Reduce the Spread of Procerum Root Disease in Christmas Tree Plantations

INTRODUCTION

Control measures against PRD have been general since so few data on the biology and means of spread of the fungus have been available. Control measures for PRD in Christmas tree plantations include: a) planting trees on sites suitable for the species, and to avoid planting white or Scotch pine on sites prone to flooding or drought, b) controlling weevils and bark beetles with insecticide sprays and cultural practices, c) removing diseased trees and their root systems, as well as, slash from within and around the plantation, d) not replanting white pine in infested areas, and e) keep weeds under control by mowing or with herbicides (Anderson and Alexander 1979; Alexander 1980; Alexander *et al.* 1988).

Weevils, including the pales weevil and the eastern pine weevil, are vectors of PRD (Chapters II - IV) therefore, there may be potential for using an insecticide as a prophylactic treatment against weevil feeding and possible spread of PRD. In the Lake States, calendar applications of lindane have been used as an effective chemical control against feeding by the root collar weevil, *Hylobius radicus* (Wilson and Millers 1983). *H. radicus* is closely related to *H. pales*, but is a primary pest because its larvae feed and develop under the bark of healthy trees disrupting water and nutrient flow (Finnegan 1959; Wilson and Millers 1983). Lindane is a highly toxic insecticide with an exceptionally long residual persistence (Ware 1983). This insecticide has many of the properties desired for a prophylactic treatment: it has long residual action, it is currently registered for use in Virginia on Christmas trees (Weidhaas *et al.* 1990) and is relatively inexpensive. Lindane is also recommended for treating stumps to reduce the number of potential breeding sites for weevils

(Weidhaas *et al.* 1990).

An alternative to insecticides is the removal of PRD-infected trees from the plantation and disposing of them by burning (Alexander and Anderson 1979, Alexander 1980). *Leptographium procerum* infected trees are a source of breeding material for weevils (Lackner and Alexander 1984; Chapter III) and weevil populations are highest in areas of Christmas tree plantations with PRD symptomatic trees (Chapter II). The removal of breeding material should reduce weevil numbers in PRD symptomatic areas as the weevils disperse to find suitable breeding material.

The effectiveness of these control measures as means of reducing the spread of PRD, however, has not been tested. Therefore, the objective of this study is to investigate the effectiveness of insecticide sprays and stump removal as means of reducing the transmission of PRD.

MATERIALS AND METHODS

Christmas tree plantations were selected in the following locations in southwestern Virginia: Floyd (Floyd County), Rt 730 (Floyd County), Stuart (Patrick County), and Riner (Montgomery County). Treatments included: a) removal of symptomatic trees within the treatment block, b) spraying lindane on the root collar of all trees within the treatment block, c) a combination of spraying lindane and removal of symptomatic trees, and d) control. There were four blocks per treatment with 20 trees per block, and treatment blocks were arranged in a randomized block design. Each tree in a treatment block was inspected for resin soaking, cankers or weevil feeding at the beginning of the test. In addition, four cores to a depth of 1-cm were taken from the cardinal directions with a #5 cork borer at the base of 20 healthy trees outside the blocks and assayed for *L. procerum*

on actidione-malt agar (AMA) (McCall and Merrill 1980). Trees in blocks treated with insecticide were sprayed with 20% EC lindane formulation at a rate of 3 l per 100 l water from the base of the stem to a height of 0.5 m until run-off, and on the ground to the drip line. Lindane treatments were applied before March 17, 1989, and March 10, 1990.

Plantations were checked monthly from June through August for trees with symptoms of PRD and the number of trees and their position in the block was recorded. PRD symptomatic trees in removal treatment blocks were disposed of monthly. For each plot, between treatment differences in the number of PRD killed trees were analyzed by a two-way ANOVA using SYSTAT version 4.1 (Systat Inc).

In May, 1989, all the trees, including the trees in the treatment plot, in the plantation at Stuart (Patrick County) were sprayed with Lindane. None of the trees had developed symptoms of PRD at this time, but in the absence of any controls this plantation was deleted from the study.

In May, 1990, two pit-fall traps, described in Chapter II, were placed in each plantation, one in a lindane treated block where trees were not removed and one in a control plot. The bolts were replaced weekly for 22 weeks. All insects were plated on AMA for 24 hours, and after 2 weeks the plates were observed for conidia of *L. procerum*.

RESULTS

None of the cores from the trees, outside of the plots, assayed for *L. procerum* yielded the fungus.

The total number of trees killed by PRD in the remaining plots is shown in Table 1. The trees were not surveyed from October, 1989 to February 1990, but only two trees in the Floyd plantation showed symptoms of PRD by March, 1990. In

1989, 5, 2 and 0.3% of all the trees showed symptoms of PRD at the Floyd, Rt 730 and Riner plantations, respectively. In 1990, 35, 15, and 4% of the remaining trees became symptomatic at the Floyd, Rt 730 and Riner plantations, respectively. In spite of the high tree mortality, none of the treatments were significantly different.

A total of 21 bark beetles, and two *P. nemorensis* were recovered from the pit-fall traps. None of the insects were contaminated with *L. procerum*.

DISCUSSION

This study was set out before the assay of apparently healthy trees in PRD symptomatic plantations, described in Chapter III was completed. The criteria used for selecting trees for the study in Chapter III were identical to that used in this study. The 20 trees examined for *L. procerum* outside of the plots in this study did not yield the fungus, however, the more extensive examination in Chapter III revealed that between 5 - 35% of apparently healthy trees in PRD symptomatic areas are infected with *L. procerum*. Approximately the same proportion of trees were killed by *L. procerum* during the 2 years of this study. For trees over five-years-old, a 2 year incubation period before disease symptoms are expressed may be possible. Wingfield (1983), noted that 12 months after inoculation with *L. procerum* 15-year-old eastern white pine showed no foliar symptoms of disease. Horner (1985), reported similar findings when he inoculated sapling loblolly, Scotch and eastern white pine.

Other possible explanation for the results include an error in experimental technique or the loss of efficacy of the insecticide. A bioassay testing the efficacy of the lindane application would have shown how long lindane was effective at killing the weevils. This was not conducted during the study since the high concentration of insecticide used was shown to give year long control against weevil oviposition in

stumps (Deboo and Weidhaas 1965). If the insecticide did not give protection throughout the summer weevils may have transmitted *L. procerum* by feeding at the base of the tree in the late summer and early fall while the insects were still active. No weevil feeding was noted on the lindane-treated trees killed by PRD in 1989 or 1990; however, future insecticide trials with lindane should include more than one application to avoid this possibility.

Table A-1. The number of trees killed by procerum root disease in a randomized block design with 4 blocks per treatment. Treatments: dead trees removed, lindane application, dead trees removed and lindane application, and control.

Plot/ Year	No. of dead trees per treatment			
	Control	Trees removed	Lindane application	Tree removed & lindane application
Floyd				
1989	0[1]	5	4	6
1990	26	29	23	27
Rte 730				
1989	2	1	1	2
1990	11	20	12	3
Riner				
1989	0	1	0	0
1990	0	1	6	9

[1] 20 trees per block

APPENDIX B

Location of Christmas tree plantations and the number and species of insects recovered from procerum root disease symptomatic plantations and asymptomatic plantations, 1988 - 1990.

Christmas tree plantations with PRD symptomatic eastern white pine were located in the following locations in southwestern Virginia: PRD1/APRD1 Floyd (Floyd County), PRD2/APRD2 Riner (Montgomery County), PRD3/APRD3 Pilot (Montgomery County), PRD4/APRD4 Pilot (Montgomery County), PRD5/APRD5 Pilot (Montgomery County), PRD6/APRD6 Pilot (Montgomery County), PRD7/APRD7 Pilot (Montgomery County), PRD8/APRD8 Riner (Montgomery County), PRD9/APRD9 Max Meadows (Wythe County), and PRD10/APRD10 Rte 617 (Floyd County). Christmas tree plantations with no symptoms of PRD were located in the following locations in southwestern Virginia: APLAN1 Rte 8 (Floyd County), APLAN2 near blue ridge parkway (Patrick County), APLAN3 near blue ridge parkway (Patrick County), APLAN4 Rte 730 (Floyd County), APLAN5 Rte 8 (Floyd County).

Plots were placed in the headlands outside the following plantations in 1990: HDLDS1-APLAN3 near blue ridge parkway (Patrick County), HDLDS2-PRD7/APRD7 Pilot (Montgomery County), HDLDS3-PRD9/APRD9 Max Meadows (Wythe County), HDLDS4-PRD10/APRD10 Rte 617 (Floyd County), HDLDS5-APLAN5 Rte 8 (Floyd County), HDLDS6-APLAN4 Rte 730 (Floyd County).

The forest plots were placed in the following locations FPL1/FPL2 were near Rte 705 (Floyd County) and FPL3 was Max Meadows (Wythe County),

Table B-1a. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in symptomatic areas of procerum root disease symptomatic plantations in 1988.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
PRD1	2	0	0	11	2	0	15	0	0
PRD2	17	12	1	6	1	0	10	6	0
PRD3	7	7	0	4	0	0	36	0	3
PRD4	12	8	0	0	0	0	9	0	0
PRD5	13	12	0	8	0	0	16	0	6
PRD6	7	7	0	3	0	0	0	0	0
PRD7	19	16	0	6	2	0	13	0	0
PRD8	2	2	0	1	0	0	7	0	0
PRD9	11	8	0	6	1	0	43	0	0
PRD10	8	6	0	4	1	0	4	0	0

[1] L. p. - Leptographium procerum

[2] Ce spp - Ceratocystis/Ophiostoma spp.

Table B-1b. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in asymptomatic areas of procerum root disease symptomatic plantations in 1988.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APRD1	3	2	0	11	0	0	9	0	1
APRD2	13	8	0	4	2	0	3	0	0
APRD3	3	1	0	6	1	0	0	0	0
APRD4	0	0	0	8	1	0	1	0	0
APRD5	1	0	0	9	1	0	1	0	0
APRD6	3	3	0	17	6	0	4	0	0
APRD7	2	1	0	14	3	0	3	0	0
APRD8	0	0	0	1	0	0	0	0	0
APRD9	11	5	0	7	1	0	34	0	0
APRD10	15	12	0	8	3	0	25	0	0

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

Table B-1c. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 5 plots in plantations with no procerum root disease in 1988.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APLAN1	2	1	0	21	4	0	6	1	1
APLAN2	0	0	0	1	0	0	0	0	0
APLAN3	1	0	0	5	1	0	8	0	0
APLAN4	0	0	0	2	0	0	0	0	0
APLAN5	11	8	0	17	2	0	9	0	0

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

Table B-2a. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in symptomatic areas of procerum root disease symptomatic plantations in 1989.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
PRD1	0	0	0	2	1	0	12	0	0
PRD2	48	46	4	0	0	0	6	0	0
PRD3	21	18	2	1	1	0	12	0	0
PRD4	6	5	1	4	2	0	1	0	0
PRD5	8	7	0	1	1	1	0	0	0
PRD6	10	9	2	2	0	0	0	0	0
PRD7	13	13	0	1	1	0	0	0	0
PRD8	5	5	1	0	0	0	0	0	0
PRD9	25	23	2	1	0	0	4	0	2
PRD10	16	12	1	2	0	0	1	0	1

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

Table B-2b. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in asymptomatic areas of procerum root disease symptomatic plantations in 1989.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APRD1	1	1	0	4	0	0	26	0	0
APRD2	10	10	0	0	0	0	0	0	0
APRD3	5	4	1	3	1	0	1	0	0
APRD4	1	1	0	3	0	0	1	0	0
APRD5	21	19	3	2	0	0	0	0	0
APRD6	5	4	0	5	2	1	4	1	1
APRD7	6	3	0	2	0	0	0	0	0
APRD8	0	0	0	0	0	0	0	0	0
APRD9	10	7	3	3	0	0	0	0	0
APRD10	3	2	0	7	0	0	2	0	0

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

Table B-2c. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 5 plots in plantations with no procerum root disease in 1989.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APLAN1	12	11	3	11	3	1	0	0	0
APLAN2	2	2	0	1	0	0	1	0	0
APLAN3[3]	17	12	0	5	3	0	19	0	0
APLAN4	2	1	2	4	0	2	20	2	0
APLAN5	5	3	0	7	1	2	1	0	0

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

3 procerum root disease symptomatic trees appeared in Aug. 1989.

Table B-3a. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in symptomatic areas of procerum root disease symptomatic plantations in 1990.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
PRD1	1	1	0	0	0	0	2	0	0
PRD2	13	10	0	8	2	0	1	0	1
PRD3	7	5	0	3	0	1	0	0	0
PRD4	7	5	0	4	0	0	1	1	0
PRD5	4	3	1	3	1	0	0	0	0
PRD6	7	6	0	3	0	0	26	2	5
PRD7	5	5	0	4	2	0	0	0	0
PRD8	0	0	0	1	0	0	6	3	6
PRD9	24	19	0	2	0	0	17	5	4
PRD10	9	6	0	1	1	0	0	0	0

[1] L. p. - Leptographium procerum

[2] Ce spp - Ceratocystis/Ophiostoma spp.

Table B-3b. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 10 paired-plots in asymptomatic areas of procerum root disease symptomatic plantations in 1988.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APRD1	0	0	0	0	0	0	5	0	0
APRD2	3	2	0	6	2	1	0	0	0
APRD3	0	0	0	6	0	0	0	0	0
APRD4	0	0	0	4	1	0	10	1	1
APRD5	7	5	0	3	1	0	0	0	0
APRD6	6	4	0	2	0	0	25	0	9
APRD7	0	0	0	1	0	0	0	0	0
APRD8	4	3	0	6	1	0	6	0	0
APRD9	1	1	0	1	0	0	0	0	0
APRD10	0	0	0	4	0	0	0	0	0

[1] L. p. - Leptographium procerum

[2] Ce spp - Ceratocystis/Ophiostoma spp.

Table B-2c. The number of insects recovered and the numbers contaminated with pathogenic fungi recovered from baited pit-fall traps in 5 plots in plantations with no procerum root disease in 1990.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
APLAN1	0	0	0	9	0	1	19	0	8
APLAN2	5	3	0	2	0	0	3	2	0
APLAN3[3]	-	-	-	-	-	-	-	-	-
APLAN4	0	0	0	4	2	0	0	0	0
APLAN5	2	1	1	3	0	1	7	0	6

1 L. p. - Leptographium procerum

2 Ce spp - Ceratocystis/Ophiostoma spp.

3 procerum root disease symptomatic trees in plantation.

Table B-4. Mean number of insects recovered in 1990 from baited pit-fall traps and the percentage contaminated with L. procerum in individual plots randomly placed in the headlands of 6 Christmas tree plantations, in 3 mixed eastern white and Virginia pine stands, and one plot in an urban setting placed where 20-year-old eastern white pine had been killed by procerum root disease.

	Insect Species								
	H. pales			P. nemorensis			Bark beetles		
Plot	Total	L. p.[1]	Ce spp.[2]	Total	L. p.	Ce spp.	Total	L. p.	Ce spp.
Headlands									
HDLDS1	16	7	0	6	0	0	4	1	0
HDLDS2	33	32	0	1	0	0	0	0	0
HDLDS3	17	12	1	10	1	3	1	0	0
HDLDS4	2	0	0	0	0	0	0	0	0
HDLDS5	4	2	1	2	0	0	0	0	0
HDLDS6	1	0	1	3	0	0	0	0	0
Forest									
FPL1	4	2	0	0	0	0	2	0	0
FPL1	4	4	0	0	0	0	0	0	0
FPL1	8	5	0	1	0	0	1	0	0

[1] L. p. - Leptographium procerum

[2] Ce spp - Ceratocystis/Ophiostoma spp.

APPENDIX C

The number of trees that insects and pathogenic fungi were recovered from healthy and procerum root disease symptomatic eastern white pines, 1988 - 1990.

Table C-1. Number of healthy and procerum root disease symptomatic eastern white pines from which insects and pathogenic fungi were recovered in 1988.

Plot/ Symptom class	Number of trees								
	Weevil larvae	Weevil larvae and *L. procerum*	Bark beetles	Bark beetles and *L. procerum*	*L. procerum*	*L. serpens*	*Ophiostoma piceae*	*Ophiostoma/ Ceratocystis* spp.	*Ophiostoma/ Ceratocystis* spp. and *L. procerum*
PRD1									
Green	0 (0)[1]	0	0	0	0	0	0	0	0
Presymptomatic green	- -	-	-	-	-	-	-	-	-
Symptomatic green	2 (16)	1	0	0	1	0	0	0	0
Symptomatic yellow	3 (21)	1	1	0	2	0	0	2	1
Symptomatic red	2 (8)	0	1	0	0	0	0	0	0
PRD5									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	- -	-	-	-	-	-	-	-	-
Symptomatic green	2 (26)	1	0	0	1	0	0	1	0
Symptomatic yellow	2 (6)	1	3	2	2	0	0	1	0
Symptomatic red	3 (27)	1	1	0	1	0	0	0	0
PRD8									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	- -	-	-	-	-	-	-	-	-
Symptomatic green	1 (15)	1	1	0	1	0	0	0	0
Symptomatic yellow	2 (7)	0	2	0	0	0	0	0	0
Symptomatic red	3 (8)	1	1	1	1	0	0	0	0
PRD9									
Green	0 (0)	0	0	0	0	0	0	0	0

Presymptomatic green	- -	-	-	-	-	-	-	-	-
Symptomatic green	1 (7)	1	1	1	3	0	0	0	0
Symptomatic yellow	2 (7)	2	1	1	4	0	0	0	0
Symptomatic red	2 (11)	1	2	0	2	0	0	1	0

[1] numbers in parenthesis indicate number of weevil larvae.

Table C-2. Number of healthy and procerum root disease symptomatic eastern white pines from which insects and pathogenic fungi were recovered in 1989.

Plot/ Symptom class	Number of trees								
	Weevil larvae	Weevil larvae and *L. procerum*	Bark beetles	Bark beetles and *L. procerum*	*L. procerum*	*L. serpens*	*Ophiostoma piceae*	*Ophiostoma*/ *Ceratocystis* spp.	*Ophiostoma*/ *Ceratocystis* spp. and *L. procerum*
PRD1									
Green	0 (0)[1]	0	0	0	0	0	0	0	0
Presymptomatic green	0 (0)	0	0	0	0	0	0	1	0
Symptomatic green	1 (14)	0	0	0	0	0	0	1	0
Symptomatic yellow	3 (27)	0	2	0	1	0	0	2	1
Symptomatic red	1 (1)	0	1	0	0	0	1	2	0
PRD5									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	1 (2)	0	0	0	2	0	0	0	0
Symptomatic green	1 (8)	1	0	0	3	0	0	1	1
Symptomatic yellow	2 (40)	1	1	1	1	0	0	0	0
Symptomatic red	2 (14)	1	0	0	3	0	0	1	0
PRD8									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	0 (0)	0	0	0	0	1	1	0	0
Symptomatic green	3 (58)	0	0	0	0	0	0	1	0
Symptomatic yellow	3 (27)	1	1	0	1	0	1	1	1
Symptomatic red	3 (19)	0	2	0	0	2	0	1	0
PRD9									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	0 (0)	0	0	0	3	0	0	0	0

Symptomatic green	2 (23)	2	2	2	3	0	0	2	2
Symptomatic yellow	3 (9)	3	2	2	3	0	0	1	1
Symptomatic red	2 (8)	2	2	2	4	0	0	2	2

[1] numbers in parenthesis indicate number of weevil larvae.

NOTE: PRD8-Y2: 2 P. nemorensis adults recovered - 2 had L. procerum
PRD8-R2: 1 P. nemorensis adult recovered - did not have L. procerum
PRD5-Y3: 2 H. pales adults - 2 had L. procerum

Table C-3. Number of healthy and procerum root disease symptomatic eastern white pines from which insects and pathogenic fungi were recovered in 1990.

Plot/ Symptom class	Number of trees								
	Weevil larvae	Weevil larvae and *L. procerum*	Bark beetles	Bark beetles and *L. procerum*	*L. procerum*	*L. serpens*	*Ophiostoma piceae*	*Ophiostoma/ Ceratocystis* spp.	*Ophiostoma/ Ceratocystis* spp. and *L. procerum*
PRD1									
Green	0 (0)[1]	0	0	0	1	0	0	2	1
Presymptomatic green	0 (0)	0	0	0	3	0	0	1	0
Symptomatic green	1 (3)	1	1	0	3	0	0	2	1
Symptomatic yellow	1 (8)	1	1	0	2	0	0	2	0
Symptomatic red	3 (14)	0	1	0	0	0	0	4	0
PRD5									
Green	0 (0)	0	0	0	1	0	0	0	0
Presymptomatic green	0 (0)	0	0	0	5	0	0	1	1
Symptomatic green	4 (55)	1	5	2	2	0	0	3	0
Symptomatic yellow	5 (77)	3	1	1	3	0	0	3	0
Symptomatic red	4 (34)	2	1	1	2	0	0	5	2
PRD8									
Green	0 (0)	0	0	0	0	0	0	0	0
Presymptomatic green	2 (16)	0	0	0	3	0	1	1	1
Symptomatic green	2 (22)	0	2	0	1	1	0	0	0
Symptomatic yellow	5 (68)	2	2	0	2	0	0	2	1
Symptomatic red	2 (32)	1	2	2	3	0	0	2	0
PRD9									
Green	0 (0)	0	0	0	0	0	0	1	0
Presymptomatic green	1 (4)	0	0	0	5	0	1	3	3

Symptomatic green	2 (23)	2	1	1	6	0	0	0	0
Symptomatic yellow	5 (70)	4	1	1	4	0	0	0	0
Symptomatic red	4 (70)	3	1	0	3	0	0	2	1

[1] numbers in parenthesis indicate number of weevil larvae.

NOTE: PRD5-R4: 6 P. nemorensis adults - recovered - 6 had L. procerum
PRD8-R5: 7 P. nemorensis adults - none had L. procerum
PRD8-Y5: 4 P. nemorensis adults - 1 of 4 had L. procerum

VITAE

Ralph John Leslie Nevill was born in Vancouver, British Columbia on November 18, 1953. In June, 1971 he received his high school diploma from Alpha Senior Secondary School in Burnaby, British Columbia. Studies in Civil Engineering Technology were completed at British Columbia Institute of Technology in June 1975. From June, 1975 to March of 1977 he worked with Columbia Hydro Constructors, and in April of 1977 he began working with the British Columbia Ministry of the Environment, Parks Branch. In November, 1978 he left the Parks Branch to travel and study in Europe. While in Europe he studied one year a Folk-Highschool in Denmark and completed the first year of a French immersion program in Switzerland. Upon his return to Canada in August 1981, he began studies towards a baccalaureate in Plant Science at the University of British Columbia. The B. A. was awarded in November, 1985 with a major in Plant Science. In January, 1986, studies continued at Simon Fraser University for the Master of Pest Management (M.P.M.) Degree in the Department of Biology. Requirements for the M.P.M. were completed in October of 1987 under the direction of Dr. J. H. Borden. Graduate work continued at Virginia Polytechnic Institute and State University under the direction of Dr. S. A. Alexander. The dissertation will be defended on December 14, 1990.