
Association of an Insect-Fungal Complex with Red Pine Decline in Wisconsin

K.D. KLEPZIG

K.F. RAFFA

E.B. SMALLEY

ABSTRACT. Red pine decline, characterized by an expanding circular area of dead and declining trees, is becoming increasingly prevalent in Lake States plantations. A 3-year study was conducted to determine whether any insects, fungi, and/or soil parameters were associated with this syndrome. The root collar weevil—*Hylobius radialis*, the pales weevil—*Hylobius pales*, the pitch-eating weevil—*Pachylobius picivorus*, the red turpentine beetle—*Dendroctonus valens* and *Hylastes porculus* were significantly more abundant in declining stands than in healthy *Pinus resinosa* stands. These root- and lower stem-infesting insects consistently carried *Leptographium terebrantis* and *Leptographium procerum*. Higher soil organic matter, pH and K levels were also associated with areas of mortality. Intensive root sampling revealed high levels of root mortality, staining, infestation with *Leptographium* species and extensively grafted root systems in declining red pine stands. This advancing belowground mortality precedes the aboveground symptoms of reduced radial growth, thin crown structure, and infestation by the pine engraver, *Ips pini*, and its fungal associate *Ophiostoma ips*. Colonization by the latter two species is always associated with and/or responsible for ultimate tree death. A sequence of interactions among this complex of organisms and abiotic factors is proposed as the cause of red pine decline. FOR. SCI. 37(4):1119-1139.

ADDITIONAL KEY WORDS. Bark beetles, root weevils, root graft, root disease, forest decline.

FOREST DECLINE AND MORTALITY SYNDROMES have intensified in recent years and pose a major threat in temperate ecosystems. A number of biotic and abiotic stresses, both natural or induced by industrial activities and forestry practices, have been proposed as causal factors (Manion 1981). Some of these syndromes involve complexes of closely associated, interdependent, pathogenic organisms.

Red pine decline was first reported in 1975 to affect plantation grown, 20-40-year-old red pine (*Pinus resinosa* Aiton) on a variety of soil types in Wisconsin, Michigan, and Illinois (Klepzig and Cummings-Carlson 1988, Raffa and Hall 1988). Because red pine is the most extensively planted tree species in Wisconsin plantations (Spencer et al. 1988), this syndrome may become a major problem for pulpwood production. A common symptom of red pine decline is a large circular area of dead trees (pocket), ringed by trees showing reduced diameter and height growth. Trees along the pocket margin may have thin crowns, whereas trees further from the pocket margin usually appear relatively healthy and vigorous. As the pocket enlarges more trees die. Symptoms characteristic of several insect and fungal species typically accompany declining stands. These species include various

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stem and root-infesting beetles, and the black and blue staining *Leptographium* and *Ophiostoma* (= *Ceratocystis*) sp.

Recently several fungi in the genus *Leptographium* have received international attention as pathogens of conifers (Wingfield et al. 1988). However, attention in North America has focused on three *Leptographium* species, the diseases they are associated with, and their means of transmission. *Leptographium wageneri* (Kendrick) Wingfield, the causal organism of black stain root disease on Douglas-fir and several species of pine (Leaphart and Gill 1959, Wagener and Mielke 1961, Smith 1967, Harrington and Cobb 1983), is vectored by *Hylastes nigrinus* (Mannerheim) (Coleoptera:Scolytidae), *Pissodes fasciatus* Le Conte (Coleoptera:Curculionidae) and *Steremnius carinatus* (Boheman) (Coleoptera:Curculionidae) (Harrington 1983, Harrington et al. 1985, Witcosky et al. 1986) and its teleomorph [*Ophiostoma wageneri* (Goheen and Cobb) Harrington] is associated with *Hylastes macer* LeConte (Coleoptera:Scolytidae) (Goheen and Cobb 1980). Nonvector transmission of *L. wageneri* apparently occurs through contact of diseased and healthy roots (Wagener and Mielke 1961, Smith 1967, Landis and Helburg 1976), through continuous xylem in root grafts (Landis and Helburg 1976, Hunt and Morrison 1986) and by short distance growth through the soil (Goheen et al. 1978). *Leptographium procerum* (Kendrick) Wingfield causes procerum root disease and has been associated with *Hylobius pales* Herbst (Coleoptera:Curculionidae), *Hylobius radialis* Buchanan (Coleoptera:Curculionidae), *Hylobius rhizophagus* Millers, Benjamin and Warner (Coleoptera:Curculionidae), *Pachylobius picivorus* (Germar) (Coleoptera:Curculionidae), *Pissodes approximatus* Hopkins (Herbst) (Coleoptera:Curculionidae) (Wingfield 1983, Lewis and Alexander 1986, Rane and Tattar 1987, Raffa and Smalley 1988b), and *Dendroctonus valens* LeConte (Coleoptera:Scolytidae). Nonvector transmission of *L. procerum* may involve root contact (Lackner and Alexander 1984, Lewis et al. 1987), but soilborne propagules do not appear important in between-tree spread (Lewis et al. 1987). *Leptographium terebrantis* Barras and Perry has been associated with decline and death of *Pinus thunbergiana* Franco and *Pinus sylvestris* L. in the northeastern United States (Highley and Tattar 1985, Rane and Tattar 1987). Suspected vectors include *D. valens* (Harrington and Cobb 1983, Owen et al. 1987), *Hylurgops porosus* LeConte (Coleoptera:Scolytidae) (Mielke 1981, Harrington and Cobb 1983, Wingfield 1983) and *Dendroctonus terebrans* Olivier (Coleoptera:Scolytidae) (Barras and Perry 1971, Rane and Tattar 1987).

Based on this and on the association of various forest decline syndromes with disrupted soil nutrient relations (Bernier and Brazeau 1988, Fluckiger et al. 1986, Kazda and Zvacek 1989, Matzner and Ulrich 1985) this study sought to determine whether biotic or abiotic agents were associated with red pine decline. Preliminary observations and reports from other systems suggested that special emphasis be placed on bark beetles, root insects, and associated microorganisms as well as soil nutrient levels and pH.

METHODS

PLOT DESCRIPTIONS

Red pine plantations exhibiting decline symptoms (Table 1) were examined for characteristic symptoms of the syndrome including an actively expanding margin

TABLE 1.

Locations and characteristics of sites used in study of red pine decline in Wisconsin.

Site	County	Location	Soil	Planting date	Thinnings	Plot type(s)*
1	Dunn	T28N, R11W-Sec 18	sandy loam	1957	1980	A
2	Dunn	T29N, R11W-Sec 14	sandy loam	1948	1979, 1984	P, B
3	Dunn	T29N, R11W-Sec 11	sandy loam	1949	1976, 1982	P
4	Jackson	T22N, R3W-Sec 21	sand	1945	1979, 1984	Pre, A
5	Washburn	T42N, R10W-Sec 6	sand	1946	1976	A
6	Waupaca	T21N, R12E-Sec 8	sand	1948	1974	Pre, B, A
7	La Crosse	T18N, R6W-Sec 10	sandy loam	1953	1975, 1986	P
8	Sauk	T8N, R4E-Sec 19	sand	1945	1975	P
9	Iowa	T8N, R4E-Sec 23	sand	1945	yes	P, A
10	Columbia	T11N, R9E-Sec 8	sandy loam	1940	yes	A
11	Sauk	T8N, R3E-Sec 19	sand	1963	no	P
12	Jefferson	T5N, R16E-Sec 36	loam	1965	no	A
13	Walworth	T4N, R16E-Sec 3	sand	1954	1987	P, B

* Types of plots located at each site. P = pocket, Pre = prepocket, B = control located beyond pocket margin, A = asymptomatic control.

of declining trees. Stands with inactive regions of mortality were excluded from this study. An effort was made to establish plots along a transect from northern to southern Wisconsin. Plots were established within these stands at the pocket margin. Two types of controls were also established: control plots located in the same stand as the pocket, but beyond the pocket margin (away from the area of mortality) and control plots located in separate, but nearby asymptomatic red pine stands. One additional plot, deemed a "putative prepocket" was also established to represent areas containing the characteristic circular pattern of stunted growth but no mortality. Preliminary observations suggest that pockets frequently originate from this condition. Research plots were established in 1987 and monitored for three growing seasons, until October 1989.

INSECT ACTIVITY

Each plot contained ten pitfall traps, ten basal trunk screen traps, and ten lower stem flight traps placed at random and one Pherotech multiple funnel pheromone trap in the center of each plot.

The pitfall traps captured adult root insects as they moved through the soil and litter (Tilles et al. 1986, Hunt and Raffa 1989) and consisted of 20 cm sections of 10 cm diameter PVC plastic drain pipe with eight holes equally spaced around the pipe circumference at one end. The interior of each trap was coated with a thin layer of Fluon®, to prevent escape of trapped insects. Both ends were capped with removable plastic lids, and two holes were drilled in the bottom lid for drainage. Traps were placed so that the entrance holes were slightly above ground level. Each trap was baited with two 0.5 dram glass vials (containing 95% ethanol and turpentine respectively) and one cut red pine stem.

The basal trunk traps captured adult root insects nocturnally ascending trunks to feed (Maki 1969, Raffa and Hall 1988). The traps were constructed from

aluminum screen and steel wire to form a skirt around the base of the tree. The upper end of the trap was shaped into an inverted funnel with an inverted glass jar placed on top of the funnel. Each jar contained a freshly cut red pine stem and a piece of tissue paper.

The lower stem flight traps were designed to capture flying insects. These traps consisted of a 3.8 l plastic milk jug with three of its sides removed so that only one large flat side and two supporting columns of plastic remained. The jug was inverted and tied to the trunk approximately 25 cm above the ground, with the striking surface against the tree. Two holes were drilled into the bottom of a plastic jar coated with Fluon® which was placed so it fit tightly over the mouth of the jug. One 6-dram glass vial, with a 1.5 cm diameter mouth, was hung inside the inverted jug. In 1987 the vial was filled with turpentine only. In 1988 and 1989, based on evidence of increased attraction to turpentine/ethanol mixtures (Phillips et al. 1988), the vial was filled with a 1:1 mixture of turpentine and ethanol.

Multiple funnel pheromone traps were used to capture adult *Ips* bark beetles as they flew toward a source of aggregation pheromone. The traps were manufactured by Pherotech Inc. (Delta, British Columbia) and are described in Lindgren (1983). These traps consist of eight overlapping plastic funnels suspended vertically with a plastic collection jar fastened to the bottom. Each trap was suspended from approximately 2 m above the ground. In 1987 there was one pheromone trap per plot, the collection jar was not filled with water, and each trap was baited with racemic 50% (+)/50% (-) ipsdienol baits from Pherotech. In 1988 there were two pheromone traps per plot, one with the collection jar filled with water to reduce predation of the pine engraver—*Ips pini* Say (Coleoptera:Scolytidae)—by red checkered beetles—*Thanasimus dubius* F. (Coleoptera:Cleridae) and one with no water to provide insects for isolations. In 1989 there was one pheromone trap per plot, and its collection jar was filled with water. The baits used in 1988 and 1989 were made from 10 cm lengths of 4 × 1 mm C-flex tubing (Fisher Inc.) filled with a mixture containing 0.024 g racemic ipsdienol (Bedoukian Research Inc. Danbury, CT) per 1 ml of 95% ethanol. A field comparison conducted in early 1988 showed that trap catches did not differ significantly between the two bait types.

Pitfall, basal trunk, and flight traps were sampled and rebaited bimonthly in 1987, beginning in May, and weekly in 1988 and 1989, beginning in late April. Sampling continued until late September each year. Pheromone traps were sampled weekly all 3 years. Mean numbers of insects collected per sampling period in pockets (including the prepocket) were compared with numbers collected in the same time period in controls using the LSD procedure in SAS (SAS Institute 1982).

FUNGAL ASSOCIATES

Fungal isolations were made from field-collected insects. One to five insects of the same species were ground in a sterile glass tissue homogenizer with 9 ml of sterile water. The liquid portion of the homogenate was removed and set aside. Another 9 ml of sterile water were added to the insect parts remaining in the homogenizer and the process repeated. The above procedure was repeated three times. Both the liquid and insect parts were saved in the final dilution. Each of the four

resulting homogenates were run through five 1/10 dilutions to give a total of four sets of six tubes each, ranging in dilution from 10^{-1} to 10^{-6} . Each tube was shaken, 0.5 ml removed from each tube and spread across the surface of a 9 cm plastic petri plate containing potato dextrose agar amended with approximately 150 ppm novobiocin (to inhibit bacterial growth). Plates were incubated at 20°–25°C under fluorescent lighting for approximately 2 weeks.

Fungal counts were made by selecting the plate at the highest concentration within a dilution series which contained countable fungal colonies. The number of colonies of each species was recorded, multiplied by the appropriate dilution factor and divided by the number of insects in the sample to estimate the number of colony forming units (CFU) per insect.

SOIL PARAMETERS

Soils were sampled in all plots in June 1988. At each stand, two 15 × 30 m rectangular grids were established, and 15 sampling points were randomly selected within each grid. In declining stands, one grid was located within the pocket itself and the other was located 10–20 m away. In control stands one grid was located within the trapping plot, the other 10–20 m away. After the litter layer had been scraped away, a soil probe was used to collect a soil sample to a depth of 60 cm at each sampling point. The 15 samples were combined and mixed to provide a composite sample for each plot. A standard forest soils analysis—including N, P, K, Ca, Mg, pH, and organic matter—was performed by the University of Wisconsin–Madison Soil and Plant Analysis Lab. Soil parameters were compared among plots using the LSD procedure in SAS. In addition, soil characteristics such as depth to water table and soil texture were qualitatively examined during the root sampling.

ROOT CONDITION

Root samples were collected from trenches running through two sites in 1987 and five sites in 1988. Trenches were dug with a backhoe to a depth of approximately 1.2 m. The 1987 sampling was conducted to yield general information about the occurrence and pattern of root mortality and fungal infection in pockets. Based on these preliminary results, a more narrowly defined sampling scheme was employed in 1988. Root samples were collected from a total of one prepocket, two control, and four declining stands.

In 1987 two trenches were dug at each of two sites (Table 1—sites 8 and 9). One trench began in the center of the pocket, and extended approximately 18 m into the living trees. The other trench started at the pocket margin and extended tangentially 3 m into apparently healthy trees on both sides of the pocket in each case. Root samples were removed at 3 m intervals. At each sampling location, a 90-cm² rectangular area of soil was removed, and any roots within this area saved so that all roots contained within the 90-cm² × 1.2 m column of soil were collected.

In 1988 four trenches were dug at two pockets (Table 1—sites 7 and 11) and one prepocket (Table 1—site 4). The trenches began 3 m inside the pocket

opening, crossed the pocket margin, and extended 9 m outside the pocket margin into apparently healthy trees. Samples were collected at 3 m inside the pocket opening (among the dead trees), at the pocket margin (designated as 0 m), and 1.5 m, 3 m, 6 m, and 9 m outside the pocket margin into apparently healthy trees. Root samples were collected by tracing a 20 cm × 25 cm area on the soil surface. The soil within this area was gently removed, to sampling depths of 0–15 cm, 15–30 cm, 30–60 cm, and 60–120 cm. Any roots within the 20 × 25 cm area down to these depths were collected. Root samples were collected in similar fashion at two healthy stands (Table 1—near site 4 and near site 9). At site 11 (Table 1) some minor modifications of the sampling scheme were necessary. All root samples were stored at 4°C.

Root segments were washed, blotted dry, their lengths and diameters measured, and their condition determined by removing the bark with a scalpel so that the phloem and xylem could be examined. Segments with light-colored, intact, succulent phloem and xylem were classified as alive. Segments with dark, detached, deteriorating phloem and discolored, dry xylem were classified as dead. Segments with gray to black phloem and or xylem were classified as stained.

Data were recorded as proportion of total root sample volume either dead, stained, or alive. For purposes of analysis, the data were transformed by taking the arcsine of the square root of the raw proportions and analyzed using the General Linear Models procedure in SAS.

In 1988, isolations were attempted from all “stained” roots, as well as subsamples of “dead” and “alive” roots, deteriorated wood residue, and insect frass. Root segments were incubated in plastic bags at 4°C for 2–3 wk. A flame-sterilized scalpel was used to peel the bark off a portion of the root segment, and the exposed tissue was surface sterilized with 70% ethanol. Three small chips were cut through the phloem and xylem and placed in 9 cm glass petri plates containing water agar amended with 50 ppm streptomycin-sulfate. In addition to the wood chips, samples of frass and residue were placed on *O. wageneri* selective medium (Hicks et al. 1980), which included cyclohexamide included to inhibit growth of fungi other than *Ophiostoma* and *Leptographium* species, and streptomycin-sulfate to inhibit bacteria. Plates were incubated at room temperature under fluorescent lighting for 2 wk and checked for fungal growth. Fungi were either identified immediately or transferred by hyphal tipping to PDA for identification.

ABOVEGROUND SYMPTOMOLOGY

Crown condition, levels of insect damage, and radial growth rates were determined for the trees nearest root sampling locations at each plot. Fullness of crown was ranked as thin, medium, or full. Damage caused by insects was determined by direct observation. Infestation and damage caused by *H. radialis* were estimated by scraping soil away from the root collar and estimating the percent of the circumference showing the characteristic tunneling wounds and black pitchy soil. The presence and number of *D. valens* infestations were estimated by counting the number of pitch tubes found around the circumference of each tree in the lower 1 m of the trunk and immediately below the soil line. Infestation by *I. pini* was diagnosed by searching the bark surface at 1.5 m height for entrance and emergence holes.

Increment cores were collected at 1.5 m height from the trees used in the estimates of crown condition and insect damage. Yearly incremental growth was measured using an ocular micrometer and periodic growth ratios calculated by dividing the most recent 5 years of growth by growth during the 5 prior years (Mahoney 1978).

RESULTS

INSECT ACTIVITY

The incidence of red pine decline was associated with increased numbers of several insect species (Table 2). All nine species monitored were more abundant in symptomatic stands, but five species of root and lower-stem feeding insects were significantly more abundant than they were in asymptomatic controls: *Hylobius radialis*, *H. pales*, *P. picivorus*, *D. valens*, and *Hylastes porculus* Erichson (Coleoptera:Scolytidae). Based on the pooled data (Table 2a) the insects captured could be divided into three groups. Three species of root weevil—*H. radialis*, *H. pales*, and *P. picivorus*—were most abundant within the immediate vicinity of the pocket. However, only *P. picivorus* densities were significantly higher in plots located at pocket margins than in plots beyond pocket margins. All three species were significantly more abundant in plots near pockets than in asymptomatic controls. A second group of insects was most abundant in plots beyond the pocket margin. Two lower trunk and root breeding bark beetles—*D. valens* and *H. porculus*—were more abundant in plots beyond the pocket margins than in plots near pockets or in asymptomatic controls. The third group of insects consisted of a root weevil and three stem colonizing species which were not significantly associated with red pine mortality. Numbers of *H. rhizophagus*, *I. pini*, *Ips grandicollis* Eichhoff (Coleoptera:Scolytidae), and *Pissodes* sp. did not significantly differ with respect to plot type. However, *I. pini* were present in high but variable densities, whereas *H. rhizophagus* and *Pissodes* sp. were relatively scarce. The low numbers of *I. grandicollis* reflect our choice of ipsdienol rather than ipsenol (its principle pheromone component) for population monitoring. However, preliminary studies show that this insect was not common, except in the more southeasterly plots.

The insect data were remarkably consistent on a year-to-year basis. The same general trends found in the pooled data were reflected in most of the trapping years. Differences in insect numbers were also found based on trapping year. *Hylobius radialis* occurred at higher levels in 1987 than in 1988 or 1989, while *H. pales* populations appeared to rise in 1988 and 1989 when compared to 1987. These differences may have been due to the severe drought in 1988 which reduced *H. radialis* throughout most of the region (Rieske and Raffa 1991). *Ips pini*, which was most abundant in 1989, appears to have benefitted from the 1988 drought.

Flight traps baited with a 1:1 turpentine/ethanol mixture captured 60× the number of *D. valens* caught with turpentine alone in 1987. Because injury data were equivalent between 1987 and 1988/1989, and the capture rates were so dramatically increased, a synergism between the two volatiles is strongly suggested as has been previously reported (Phillips et al. 1988). The funnel traps

TABLE 2.

Mean numbers of insects collected per sampling period from red pine plantations in Wisconsin in 1987, 1988, and 1989 pooled (a), 1987 (b), 1988 (c) and 1989 (d). Means followed by same letter within a row are not significantly different ($P < 0.05$); * = symptomatic stands significantly different from asymptomatic control stands ($P < 0.05$); ns = symptomatic stands not significantly different from asymptomatic control stands ($P < 0.05$); — = insect species not sampled that year.

Insect	Symptomatic stands			
	Asymptomatic stands	Plot location		Symptomatic pooled
		Beyond pocket margin	At pocket margin	
a. 1987, 1988 & 1989 pooled				
<i>H. radialis</i>	0.20 (0.91) ^a	0.53 (2.18) ^{ab}	0.65 (1.76) ^b	0.62 (1.89)*
<i>H. pales</i>	3.72 (7.51) ^a	5.22 (7.45) ^{ab}	6.66 (11.77) ^b	6.24 (10.69)*
<i>P. picivorus</i>	0.04 (0.24) ^a	0.28 (0.89) ^a	1.24 (3.49) ^b	0.96 (3.00)*
<i>D. valens</i>	8.39 (25.63) ^a	34.28 (117.29) ^b	14.51 (70.92) ^a	20.36 (87.52) ^{ns}
<i>H. porculus</i>	3.51 (8.65) ^a	9.52 (19.23) ^b	6.91 (14.99) ^{ab}	7.67 (16.34)*
<i>H. rhizophagus</i>	0.01 (0.08) ^a	0.04 (0.18) ^a	0.04 (0.20) ^a	0.04 (0.20) ^{ns}
<i>Pissodes</i> sp.	1.33 (4.96) ^a	2.06 (4.90) ^a	1.22 (3.86) ^a	1.50 (4.24) ^{ns}
<i>I. pini</i>	11.22 (29.66) ^a	16.69 (35.42) ^a	16.86 (35.82) ^a	16.81 (35.65) ^{ns}
<i>I. grandicollis</i>	1.39 (5.27) ^a	2.34 (5.29) ^a	1.81 (4.48) ^a	1.97 (4.72) ^{ns}
b. 1987				
<i>H. radialis</i>	0.00 ^a	1.87 (4.47) ^b	1.82 (3.42) ^{ab}	1.74 (3.71)*
<i>H. pales</i>	0.16 (0.37) ^a	0.70 (0.00) ^{ab}	1.16 (2.00) ^b	0.98 (1.80)*
<i>P. picivorus</i>	0.00 ^a	0.00 ^a	0.10 (0.37) ^a	0.19 (0.98) ^{ns}
<i>D. valens</i>	0.11 (0.32) ^a	1.91 (2.72) ^b	1.19 (2.42) ^{ab}	1.33 (2.42)*
<i>H. porculus</i>	—	—	—	—
<i>H. rhizophagus</i>	0.00 ^a	0.00 ^a	0.04 (0.20) ^a	0.06 (0.29) ^{ns}
<i>Pissodes</i> sp.	—	—	—	—
<i>I. pini</i>	0.21 (0.92) ^a	13.65 (41.47) ^a	5.71 (16.42) ^a	8.42 (27.36) ^{ns}
<i>I. grandicollis</i>	—	—	—	—
c. 1988				
<i>H. radialis</i>	0.36 (1.32) ^a	0.14 (0.41) ^a	0.43 (0.96) ^a	0.34 (0.83) ^{ns}
<i>H. pales</i>	2.72 (4.09) ^a	5.41 (8.69) ^b	7.29 (12.25) ^b	6.67 (11.20)*
<i>P. picivorus</i>	0.01 (0.12) ^a	0.24 (0.75) ^a	1.04 (2.76) ^b	0.78 (2.32)*
<i>D. valens</i>	7.78 (23.33) ^a	59.78 (168.67) ^b	26.16 (110.16) ^{ab}	37.29 (132.78) ^{ns}
<i>H. porculus</i>	0.92 (2.38) ^a	5.78 (18.11) ^b	4.39 (13.09) ^{ab}	4.85 (14.89)*
<i>H. rhizophagus</i>	0.04 (0.20) ^a	0.10 (0.31) ^a	0.08 (0.31) ^a	0.09 (0.31) ^{ns}
<i>Pissodes</i> sp.	1.33 (4.96) ^a	2.06 (4.90) ^a	1.22 (3.86) ^a	1.50 (4.24) ^{ns}
<i>I. pini</i>	4.62 (13.61) ^a	11.55 (32.30) ^a	7.68 (17.21) ^a	8.96 (23.37) ^{ns}
<i>I. grandicollis</i>	0.62 (2.27) ^a	1.06 (3.56) ^a	0.64 (1.44) ^a	0.78 (2.36) ^{ns}
d. 1989				
<i>H. radialis</i>	0.11 (0.32) ^a	0.20 (0.47) ^a	0.32 (0.74) ^a	0.29 (0.69)*
<i>H. pales</i>	5.74 (10.27) ^a	7.94 (6.62) ^a	8.60 (13.18) ^a	8.44 (11.88) ^{ns}
<i>P. picivorus</i>	0.10 (0.34) ^a	0.55 (1.25) ^a	1.97 (4.60) ^b	1.62 (4.09)*
<i>D. valens</i>	11.28 (30.63) ^a	19.85 (29.36) ^a	9.85 (24.55) ^a	12.31 (26.07) ^{ns}
<i>H. porculus</i>	6.22 (11.56) ^a	14.77 (19.78) ^b	9.25 (16.28) ^{ab}	10.61 (17.30)*
<i>H. rhizophagus</i>	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^{ns}
<i>Pissodes</i> sp.	—	—	—	—
<i>I. pini</i>	21.39 (41.26) ^a	26.17 (34.48) ^a	31.13 (48.98) ^a	29.89 (45.72) ^{ns}
<i>I. grandicollis</i>	2.21 (7.13) ^a	4.20 (6.72) ^a	2.93 (5.89) ^a	3.24 (6.10) ^{ns}

baited with ipsdienol also captured *H. radialis*, *H. pales*, *P. picivorus*, *D. valens*, and *H. porculus* in addition to *Ips*. This suggests a kairomonal response to the pheromone and/or ethanol carrier, although a simple passive interception cannot be discounted.

FUNGAL ASSOCIATES

Two species of *Leptographium* were consistently isolated from the five insects associated with red pine decline (Table 3). Conversely, each insect species carried predominantly one fungus, although other staining fungi were sometimes present. *Leptographium procerum* was consistently isolated from *H. radialis* (78%), *H. pales* (80%), *P. picivorus* (71%), and occasionally from *H. porculus* (13%), and *D. valens* (7%). *Leptographium terebrantis* was often carried by *D. valens* (73%) and *H. porculus* (46%) and rarely by *H. radialis* (8%). Inoculum loads were extremely variable, ranging from 20 to 5×10^5 colony-forming units per insect. However, among those insects which frequently carried a particular fungus ($\geq 46\%$) and where sample sizes were at least 10, inoculum load was within the range of $6.9\text{--}91.8 \times 10^3$. No clear trends were exhibited between inoculum load and insect species or stand condition.

Other staining fungi isolated from collected insects included *O. ips* (from *H. radialis*, *H. pales*, *Pissodes* sp., *D. valens*, *I. pini*, *I. grandicollis*, and *H. porculus*), *Ophiostoma minus* (Hedgcock) Sydow & P. Sydow (from *H. porculus*), *Ophi-*

TABLE 3.

Staining fungi isolated from bark beetles and weevils collected from declining and asymptomatic red pine stands in Wisconsin. N = total number of insects isolated from; % Isolation = percentage of insect samples from which the given fungus was recovered; C.F.U. = colony forming units of each species (- = C.F.U.'s not calculated but fungus present); S.D. = standard deviation.

Insect	N	Fungus	% isolation	C.F.U./isolation (S.D.)
<i>H. radialis</i>	37	<i>L. procerum</i>	78	9.19×10^4 (3.50×10^5)
		<i>L. terebrantis</i>	8	6.70×10^2 (5.72×10^2)
		<i>O. ips</i>	5	5.50×10^4 (6.36×10^4)
		<i>Graphium</i> sp.	22	4.35×10^3 (7.12×10^3)
<i>H. pales</i>	10	<i>L. procerum</i>	80	9.18×10^3 (1.35×10^4)
		<i>O. ips</i>	10	3.00×10^3 (0)
		<i>Graphium</i> sp.	20	1.35×10^4 (7.78×10^3)
<i>P. picivorus</i>	7	<i>L. procerum</i>	71	2.0×10^1 (0)
<i>Pissodes</i> sp.	4	<i>O. ips</i>	50	6.50×10^2 (7.90×10^2)
<i>D. valens</i>	22	<i>L. procerum</i>	7	1.00×10^2 (0)
		<i>L. terebrantis</i>	73	6.91×10^3 (1.55×10^4)
		<i>O. ips</i>	20	6.67×10^3 (5.77×10^3)
<i>I. pini</i>	25	<i>L. procerum</i>	12	1.03×10^2 (9.50×10^1)
		<i>O. ips</i>	100	1.13×10^4 (2.12×10^4)
		<i>O. nigrocarpum</i>	8	1.50×10^2 (7.07×10^1)
<i>I. grandicollis</i>	4	<i>O. ips</i>	100	5.03×10^5 (9.98×10^5)
<i>H. porculus</i>	37	<i>L. procerum</i>	13	—
		<i>L. terebrantis</i>	46	6.91×10^3 (1.55×10^4)
		<i>O. ips</i>	3	—
		<i>O. minus</i>	5	—

stoma nigrocarpum (R.W. Davidson) De Hoog (from *I. pini*), and a *Graphium* sp. (from *H. radialis* and *H. pales*). In addition, isolations inconsistently yielded non-staining fungi such as *Trichoderma* sp., *Aspergillus* sp., and *Aureobasidium* sp. as well as a few unidentified yeasts and basidiomycetes. Most of these are considered saprophytic or may be gut symbionts of the insects. Isolations from *T. dubius* yielded the saprophytes mentioned above but no staining fungi.

SOIL PARAMETERS

Soils from declining and asymptomatic stands were generally similar. A few parameters, however, appear related to stand condition (Table 4). Samples from within areas of mortality exhibited on average, higher K levels (64.44 kg/ha) than did samples collected 10–20 m away (40.63 kg/ha). These stands in turn contained less K than did asymptomatic stands (67.95 kg/ha). Both higher pH and lower organic matter levels were associated with stands showing symptoms of red pine decline. The pH of soil samples collected from within pockets (5.28) and from areas 10–20 m away (5.32), was significantly higher than that of samples collected in asymptomatic stands (4.99). Percentage of soil organic matter was lower in soils from pockets (1.07%) and significantly lower in within-stand controls (0.98%) than in soils from asymptomatic stands (1.39%). In addition, N levels were significantly lower in within-stand controls (0.04%) than in asymptomatic stands (0.07%), but did not significantly differ from pockets (0.05%). No other significant differences were found in the soil parameters measured.

ROOT SAMPLING

Declining stands had very high root mortality and staining compared to apparently healthy stands, which had roots essentially free of staining and mortality (Figures

TABLE 4.

Analysis of pH, organic matter, and nutrient levels in composite samples from symptomatic and asymptomatic red pine stands in Wisconsin. Means followed by the same letter within a row are not significantly different ($P < 0.05$), numbers in parentheses are standard deviations.

Parameter	Asymptomatic stands	Symptomatic stands		
		Beyond pocket margin	Soil sampling location at pocket margin	Within pocket interior
	N = 8	N = 8	N = 8	N = 8
pH	4.99 (0.22) ^a	5.18 (0.08) ^{ab}	5.32 (0.40) ^b	5.28 (0.18) ^b
%O.M.	1.39 (0.21) ^a	0.98 (0.34) ^b	1.07 (0.34) ^{ab}	1.07 (0.31) ^{ab}
%N	0.07 (0.02) ^a	0.04 (0.01) ^b	0.05 (0.02) ^{ab}	0.04 (0.01) ^{ab}
kg/ha P	41.32 (39.16) ^a	31.23 (15.13) ^a	27.87 (13.58) ^a	32.22 (20.84) ^a
kg/ha K	67.95 (31.89) ^a	50.43 (8.46) ^{ab}	40.63 (11.49) ^b	64.44 (28.09) ^{ab}
kg/ha Ca	728.53 (264.54) ^a	658.48 (93.53) ^a	742.55 (223.66) ^a	658.48 (111.07) ^a
kg/ha Mg	77.05 (37.13) ^a	78.45 (25.40) ^a	114.88 (87.37) ^a	93.86 (36.40) ^a

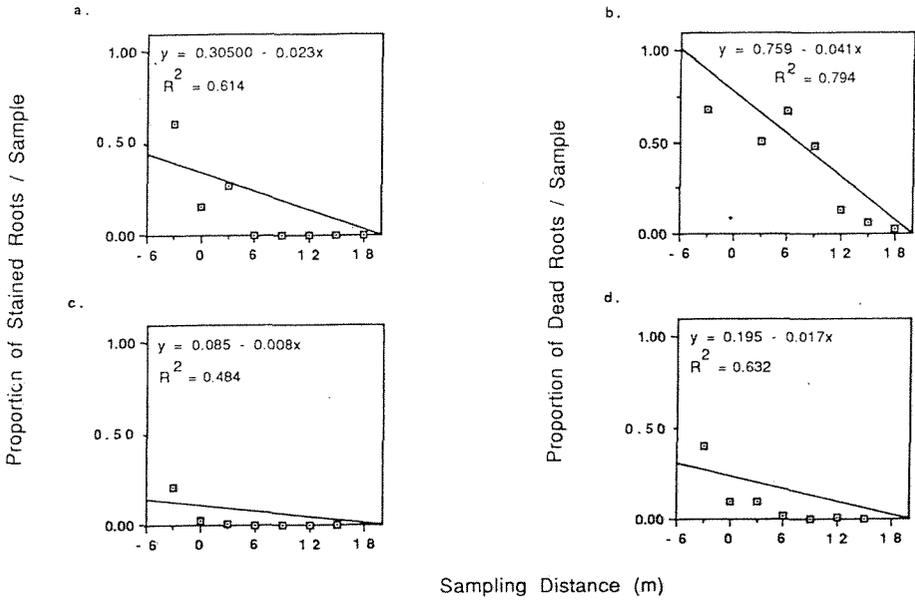


FIGURE 1. Proportion of dead and stained roots in relation to distance from the pocket margin in preliminary 1987 root sampling at site 9 (a & b) and site 8 (c & d).

1, 2, 3). Root mortality and staining in the prepocket were intermediate between pocket and control levels. Within declining stands, mortality and staining in roots were highest near the margin between declining and healthy trees, and decreased in a close to linear fashion as distance from the margin increased. Trees imme-

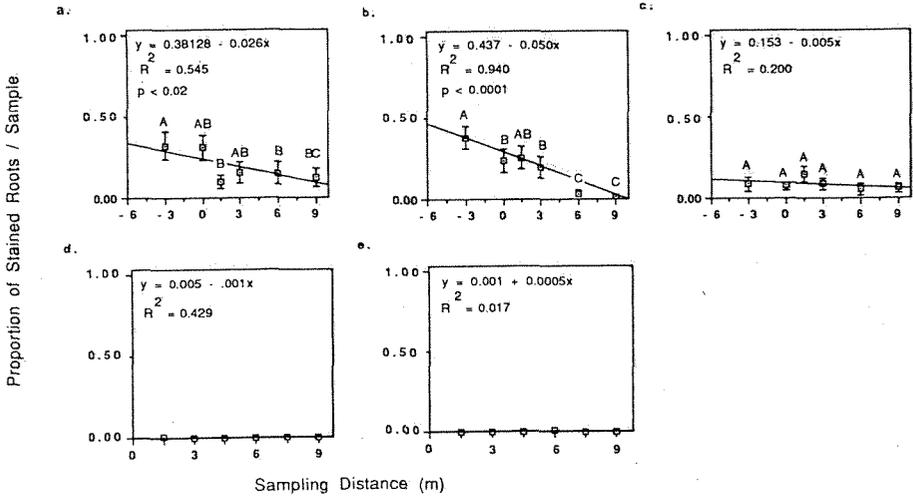


FIGURE 2. Mean proportions of stained roots in relation to sampling distance in 1988 root sampling at site 7 pocket (a), site 11 pocket (b), site 4 prepocket (c), site 9 control plot (d) and site 4 control plot (e). X-axis = distance from pocket margin (a, b, c) or from randomly chosen point within the stand (d, e). Y-axis = proportion by volume of stained roots per sample (non-transformed data). R^2 and regression equations refer to arcsine (square root) transformed data. Standard error bars are given for each mean. Means, within a plot, followed by same letter are not significantly different ($P < 0.05$) as separated by L.S.D.

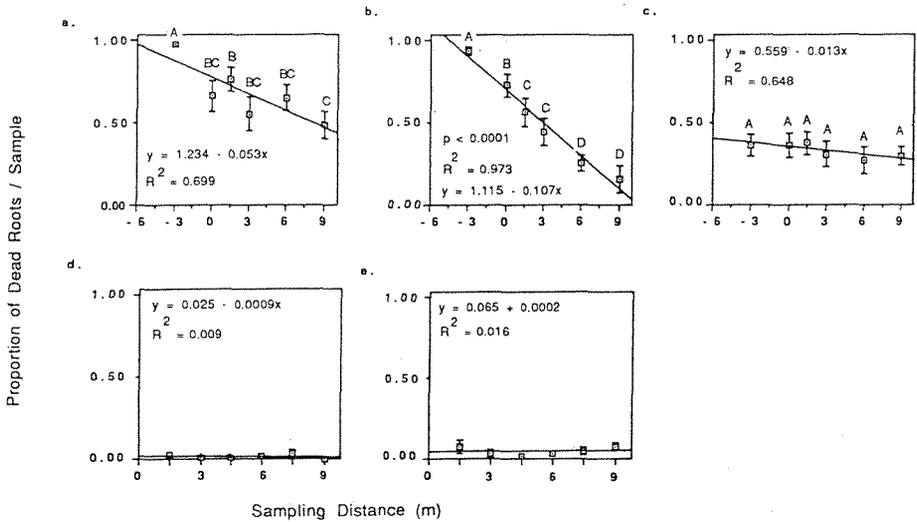


FIGURE 3. Mean proportions of dead roots in relation to sampling distance in 1988 root sampling at site 7 pocket (a), site 11 pocket (b), site 4 pre-pocket (c), site 9 control plot (d) and site 4 control plot (e). X-axis = distance from pocket margin (a, b, c) or from randomly chosen point within the stand (d, e). Y-axis = proportion by volume of dead roots per sample (non-transformed data). R^2 and regression equations refer to arcsine (square root) transformed data. Standard error bars are given for each mean. Means, within a plot, followed by same letter are not significantly different ($P < 0.05$) as separated by L.S.D.

diately beyond the range of aboveground symptoms in declining stands exhibited higher root mortality and staining than trees in asymptomatic stands.

Leptographium procerum and *L. terebrantis* were isolated from roots in declining but not asymptomatic plantations. Staining fungi isolated infrequently from roots included *O. ips*, *O. nigrocarpa*, and *Graphium* sp. *Ophiostoma minus* was found fruiting in dead, but not stained, roots from one declining stand only. Neither any *Armillaria* spp. nor any other root rotting fungus was found in abundance in declining or healthy stands. Although all stained roots were dead, not all dead roots contained stained tissue. Some live roots exhibited extensive resinosis but no staining. No staining fungi were isolated from stained roots collected more than 3 m away from the pocket margin into the living trees. This may indicate the need for a more sensitive isolation technique. Alternatively, staining at these distances may be caused by still other factors.

Hylastes porculus pupal chambers and larvae were found only within roots of trees inside pocket margins. Both *Ophiostoma huntii* (Robinson-Jeffrey) De Hoog & R.J. Scheffer and *L. terebrantis* were closely associated with damage by this insect in all three of the declining stands sampled. This represents the first occurrence of *O. huntii* in red pine in Wisconsin.

Soil characteristics such as water tables and texture did not noticeably change with distance from the pocket margin. The various stands differed in these parameters, but these differences were not correlated with presence or absence of decline. Neither root mortality nor staining differed with respect to sampling depth (according to GLM analysis) with one exception (Klepzig 1989) and thus, separation of root samples by depth was apparently unnecessary. Root grafting, both tree to tree and tree to stump, was extensive within all stands sampled.

Periodic growth ratios (PGRs) were lower near the pocket margin than amongst apparently healthy trees farther away (Figure 4). In contrast, periodic growth ratios were relatively constant across sampling distance in control plots, where belowground mortality and staining were also lacking. This corresponded with the pattern of root staining and mortality described above. Periodic growth ratios in declining stands, however, were not necessarily lower than those in control stands.

Trees with lower growth rates and high root mortality and staining also exhibited foliar decline symptoms. Thin crowned trees were mainly found near the pocket margin while full crowned trees were more prevalent away from the areas of highest root mortality (Figure 5).

Rates of *H. radialis* infestation were higher near the pocket margin at site 3 but were more variable in the other sites sampled (Figure 6). At both pockets, *D. valens* damage was only evident in living and dead trees closest to the area of mortality and apparent aboveground symptoms. *Ips pini* was closely associated with tree mortality. All dead trees were infested, and conversely, no infested trees survived. Trees infested with *H. radialis* and/or *D. valens* remained alive until colonized by *I. pini*. No damage attributable to *D. valens* or *I. pini* was observed at the prepocket or controls, despite their presence in the various traps.

DISCUSSION

Red pine decline appears to be caused by a complex of interacting organisms. Five insect species—*H. radialis*, *D. valens*, *H. pales*, *P. picivorus*, and *H. porculus*—

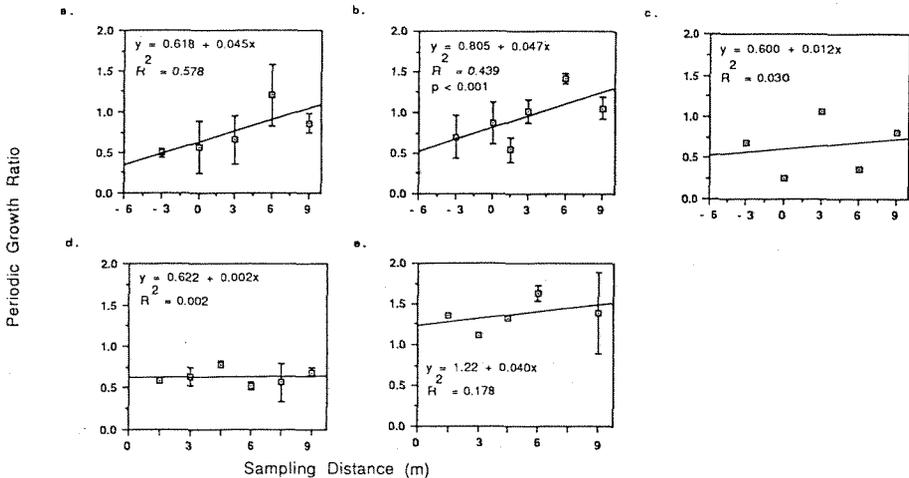


FIGURE 4. Periodic growth ratios (as measured from increment cores) of trees with respect to sampling distance at site 7 pocket (a), site 11 pocket (b), site 4 prepocket (c), site 9 control plot (d) and site 4 control plot (e). X-axis = distance from pocket margin (a, b, c) or from randomly chosen point within the stand (d, e). Standard error bars are given for each mean.

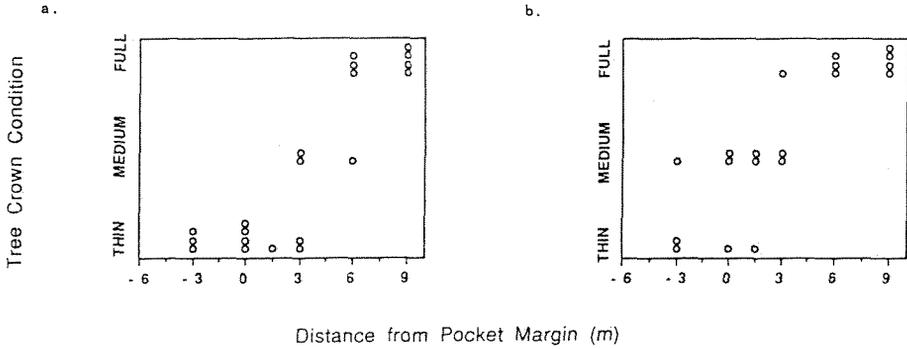


FIGURE 5. Crown condition of trees with respect to distance from the pocket margin at the site 7 pocket (a) and site 11 pocket (b).

were found to occur in higher numbers in stands containing pocket mortality than in stands without. This is similar to the increased levels of vector activity within stands associated with the incidence of black stain root disease caused by *L. wageneri* (Hansen 1978, Harrington et al. 1983). These five insects have also been demonstrated to be consistently associated with *L. procerum* and *L. terebrantis*, as well as other staining fungi, and may be serving as vectors of these fungi. Most of these insects have been reported to carry similar fungi in other disease complexes (Lackner and Alexander 1982, Raffa and Smalley 1988a, Wingfield 1983).

Leptographium procerum and *L. terebrantis* were consistently associated with declining and dead trees in affected, but not asymptomatic, stands and may be acting as pathogens. This study also provides the first report of an association between *H. porculus* and *Leptographium* or *Ophiostoma* spp. *Ophiostoma huntii* was found at both the pockets sampled in 1988 and tentatively identified in roots from the site 9 pocket in 1987. The association of *O. huntii* with *H. porculus* in red pine roots suggests that this fungus may also be important in red pine decline, especially considering that the perfect state of *L. wageneri* occurs within *H. macer* galleries (Goheen and Cobb 1978). In contrast, *O. minus* was found in only the site 7 pocket.

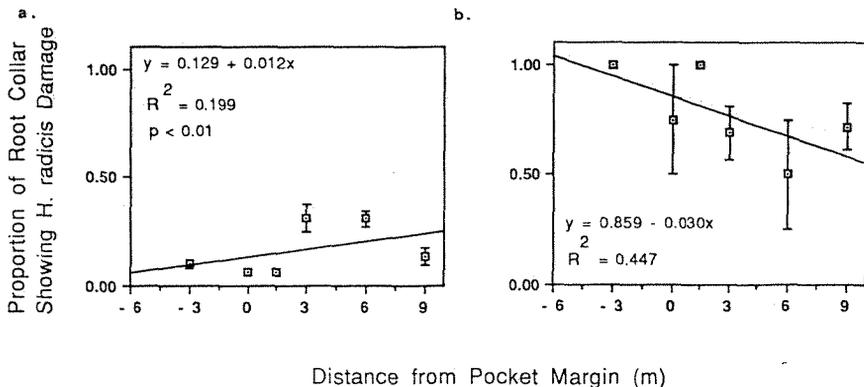


FIGURE 6. Mean level of *H. radicis* damage (black pitchy soil)/tree in relation to distance from the pocket margin at the site 7 pocket (a) and site 11 pocket (b).

Declining stands also had higher levels of organic matter, pH, and K than asymptomatic stands. Although significant, these differences in soil parameters need to be viewed in context. For example, all three mean pH and organic matter values are considered to be in the medium range, and both mean levels of K within the low range, for evergreen plantations (University of Wisconsin-Madison, Soil and Plant Analysis Laboratories analysis). There is currently no evidence that these differences could impose enough stress on trees to directly decrease growth or cause mortality. The periodic growth ratio (PGR) data also support this view. Asymptomatic regions of declining stands had PGRs no lower than those of the control plots, and one control (the site 9 control) had PGRs lower than the declining trees (Figure 4). However, the possibility that these factors may predispose trees to some or all of the biological agents associated with red pine decline cannot be dismissed. For example, soil moisture and structure have been previously associated with *Leptographium* sp. root diseases (Leaphart and Copeland 1957, Goheen et al. 1978). In our study red pine decline is closely associated with sandy soils, but sandy sites in Wisconsin are often planted to red pine so no conclusions can be drawn from this association. In the case of red pine decline, drought may serve mainly to increase the rate of expansion of pockets by creating conditions favorable for *I. pini* attack. Future study of the role of abiotic factors in this syndrome should concentrate on the relationship between the soil characteristics and weather factors identified in this study and susceptibility to root and lower-stem infesting insect-fungal complexes.

These multiple biotic agents, and perhaps mitigating abiotic factors, appear to function as an interacting complex. The association with declining red pines does not demonstrate a causal role as primary agents for any one of these organisms. For example, the composition of root- and lower-stem-feeding insects varied among pockets suggesting that no one species can solely be associated with red pine decline. Some stands contained high levels of *D. valens* and *H. porculus* but relatively low numbers of *H. radialis* and *P. picivorus*, and various combinations thereof, even though all showed an overall degree of association (Klepzig 1989). It is unnecessary, therefore, for all of the insects associated with red pine decline to be present for mortality to occur. Moreover some of these insects appear unable to colonize healthy host tissue on their own. In caging experiments, neither *D. valens* nor *H. porculus* was able to construct galleries in healthy red pine roots (Klepzig, unpublished data).

Although we found no symptomatic stands which did not contain both *L. procerum* and *L. terebrantis*, these fungi alone are probably not capable of causing the symptoms seen in declining stands. Inoculations of main stems and roots of healthy red pines with these two fungi produced resinous lesions but did not cause mortality or extensive colonization of the host (Raffa and Smalley 1988b). Neither of these two fungi have been reported to be capable of causing mortality when inoculated into mature trees.

Based on the results of this study, we propose a scenario for the initiation and spread of decline in plantation red pine (Figure 7): (1) moderately pathogenic *Leptographium* spp. introduced into a stump or stressed tree by root- and lower-stem-feeding insect vectors gain access to the communal root system; (2) by spreading through root grafts, the fungi cause increasing amounts of root mortality to adjacent trees; (3) root mortality continues, spreading in a circular pat-

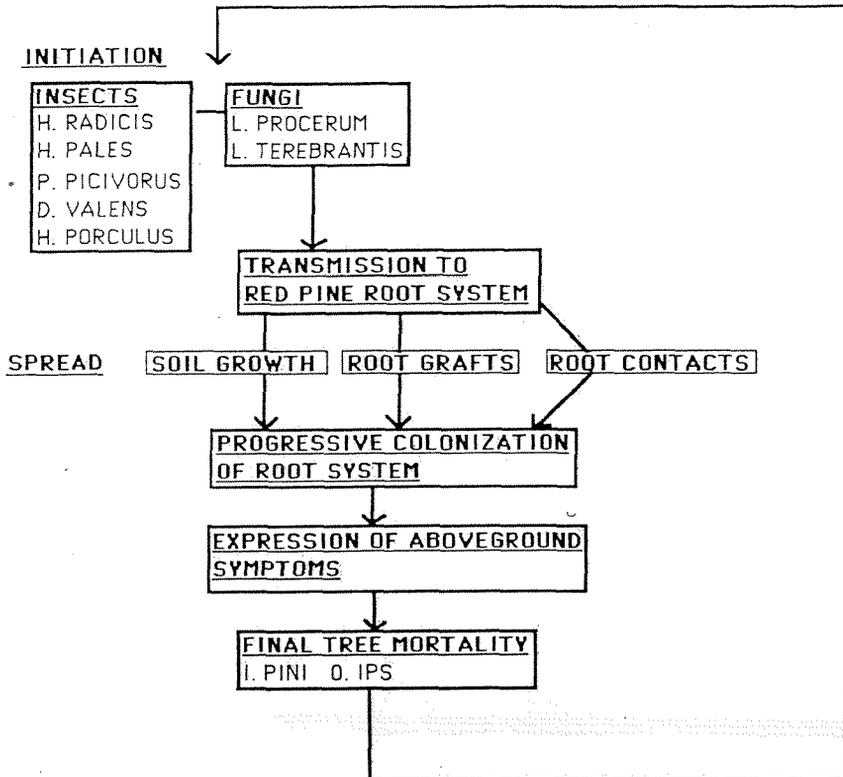


FIGURE 7. Flow diagram illustrating a proposed scenario for the etiology of red pine decline. See text for explanation.

tern, causing reduced growth in severely infected trees; (4) trees within the area of highest root mortality become sufficiently stressed to allow increased attacks of *H. radialis*, *D. valens*, and *H. porculus*, and fatal attacks by *I. pini*; (5) dead trees and stumps then become suitable for *H. pales*, *P. picivorus*, and other members of the root- and lower-stem-feeding guild, which now increase in number within pockets. These insects introduce additional inoculum, thus contributing to pocket expansion.

The role of root- and lower-stem-feeding insects as vectors of pathogenic fungi (step 1) is supported by the higher population densities of these insects in declining stands and affected trees (Table 2), the higher levels of *Leptographium* in these areas (Figures 1, 2, 3), and the consistent isolation of *Leptographium* from these insects (Table 3) and their galleries. This view is also supported by the known ability of *H. radialis* and *D. valens* to colonize living trees (Smith 1960, Wilson and Millers 1983), and the established vector relationships of related insects and fungi in other complex diseases (Goheen 1976, Harrington 1983, Lewis and Alexander 1986, Witcosky et al. 1986). Moreover, *D. valens* and *H. porculus* were most frequently found in asymptomatic regions of declining stands, suggesting an early role in pocket development. The relative importance of *H. radialis* and *D. valens* is not clear. *Hylobius radialis* damage was frequently observed in both control and declining stands, whereas *D. valens* injury was seldom

observed in control stands. This could indicate that *H. radialis* is relatively more aggressive than *D. valens* in attacking healthy trees or, conversely, that *D. valens* is more strongly associated with declines and so its attacks lead more frequently to large-scale mortality. It could also suggest that the relative importance of these two species varies with site and environmental conditions. At this time, both a definite vector relationship and a consistent sequence of infestation remain to be established.

The movement of *Leptographium* through root grafts (step 2) is supported by direct observations of excavated tissue, the extensive tree-to-tree and tree-to-stump root grafting observed in these and other studies (Stone 1974), and reports by other authors (Landis and Helburg 1976, Hessburg and Hansen 1986, Hunt and Morrison 1986). The expanding circular spread of decline is also consistent with intertree root graft spread. The possibility of fungal spread through root contacts (Wagener and Mielke 1961, Hunt and Morrison 1986) and/or short range growth through the soil (Hicks et al. 1980) cannot be dismissed. However, there are also observations of extensive defensive reactions—within healthy roots grafted to infected roots—that are successful in preventing fungal invasion of the healthy root (Goheen 1976, Klepzig, personal observations). These observations combined with observations of pockets that appear to have stopped expanding suggest that certain conditions must exist for these fungi to invade healthy roots via root grafts.

The importance of root infection and deterioration in the subsequent weakening of these trees (step 3) is supported by the prior appearance of root staining to aboveground symptoms (Figures 1, 2, 3), the overall higher rates of root injury in declining stands, and the intermediate level of staining in the prepocket. The aboveground symptoms of radial growth reduction (Figure 4) and crown thinning (Figure 5), within declining stands as compared to controls, correspond to the belowground pattern. This hypothesis is consistent with the reductions in growth before tree death (Wagener and Mielke 1961) and thin crowns (Leaphart and Gill 1959) associated with *Leptographium* spp. in other pines.

The increase in susceptibility to *H. radialis*, *D. valens*, and *H. porculus* (step 4) is suggested by their increased infestation rates in the root-infested living trees (Figure 6). That is, these insects may be both inducers and beneficiaries of a progressively deteriorating communal root system. *Dendroctonus valens*, in particular, was not observed successfully breeding in control stands. More importantly, *I. pini* attacks often follow in these trees, and successful attacks are always fatal. Living red pines frequently show evidence of successful breeding by *D. valens* and *H. radialis*, but rarely *Ips*. The possibility that root-infesting agents predispose trees to fatal *Ips* attack is supported by the decreased defensive responses to bark beetle attack observed among trees with thin crowns (Christiansen et al. 1987), low periodic growth ratios (Raffa and Berryman 1983), and stem infection (Raffa and Berryman 1982)—all factors observed in our system. Moreover, Goheen and Cobb (1980) and Wagener and Mielke (1961) observed higher frequencies of bark beetle infestation among pines with *Leptographium* root disease.

The increased breeding substrate for *H. pales* and *P. picivorus*, and their potential role as additional sources of fungal inoculum (step 5), are supported by the increased densities of these insects in declining stands (Table 2), and their

association with *L. procerum* (Table 3). These species are known to be rapid colonizers of dead tree and stump roots. Although *D. valens* and *H. radialis* may prefer and/or be more successful in stressed than dead trees because of competition with the secondary weevils, the former two species can also colonize dead tissue. The biology and behavior of *H. porculus* is poorly understood, and so its exact role is difficult to define.

Future research on red pine decline will concentrate on testing the individual components of this model. If this model is correct, control options could involve clearcutting of trees, in which all root grafts are severed and stumps removed, within and approximately 15 m beyond the pocket. Preventative options could include mixed-block planting in a staggered row fashion to prevent formation of continuous, communal root systems. A better understanding of the pathogenicity and taxonomy of the associated fungi, and the behavioral characteristics of the associated insects, should lead to a more complete understanding of this and other conifer declines.

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AUTHORS AND ACKNOWLEDGMENTS

K.D. Klepzig is with the Dept. of Entomology, 345 Russell Labs, 1630 Linden Drive, University of Wisconsin—Madison, Madison, WI 53706 and the Dept. of Plant Pathology, 484 Russell Labs, 1630 Linden Drive, University of Wisconsin—Madison, Madison, WI 53706. K.F. Raffa is with the Dept. of Entomology and E.B. Smalley is with the Dept. of Plant Pathology, University of Wisconsin—

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