AN ABSTRACT OF THE THESIS OF

Jeffrey John Witcosky for the degree of Master of Science

in ENTOMOLOGY presented on November 11, 1981

Title: Insects Associated with Black Stain Root Disease of Douglas-fir in Western Oregon.

Redacted for Privacy

Abstract approved: _____
Lee C. Ryker

The root systems of Douglas-fir trees infected with Verticicladiella wagenerii and assigned by crown color and terminal growth characteristics to several stages of decline were excavated at three widely separated sites in the Coast Range of Oregon. Data were gathered on insect species present, extent of colonization of the root system and lower stem by V. wagenerii, and the presence or absence of viable V. wagenerii inoculum, either as hyphae in wood or as conidia, within diseased trees.

The weevils Steremnius carinatus and Pissodes fasciatus and the scolytid Hylastes nigrinus were commonly associated with diseased roots and root collar-lower stem portions of diseased trees. In established pockets of V. wagenerii-induced mortality, damage by S. carinatus and P. fasciatus was always noted in trees in the year of death and H. nigrinus damage occurred in 92% of these same trees. H. nigrinus and S. carinatus, associated primarily with root damage, were the first insects to invade diseased trees. Insects sequentially colonized roots of the diseased trees as each root succumbed to infection; the colonization process spanned two to four years in most cases. P. fasciatus attack was associated with tree death.

V. wagenerii occurred as viable inoculum throughout the decline of host trees. Viable V. wagenerii hyphae were found in 75% of the trees

recently killed by the disease. Nearly 100% of all declining trees harbored viable V. wageneriiinoculum. Trees exhibiting severe crown symptoms had nearly their entire root system colonized by the pathogen. Conidiophores of V. wageneriiwere observed in galleries and pupal cells of all three beetle species. The coincidence of viable inocula and adults of these three species of root-inhabiting insects at the time of their dispersal makes H. nigrinus, P. fasciatus, and S. carinatus potential vectors of black stain root disease in Douglas-fir.

Insects Associated with Black Stain Root Disease of Douglas-fir in Western Oregon

by

Jeffrey John Witcosky

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed November 11, 1981

Commencement June 1982

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Redacted for Privacy

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Date thesis is presented November 11, 1981

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ACKNOWLEDGEMENTS

It is with sorrow that I write these few lines acknowledging the late Dr. Julius Rudinsky, my major professor. Dr. Rudinsky's guidance provided me with the enthusiasm, support, and confidence needed to develop and carry out this thesis research. His presence is sorely missed; forest entomologists have lost a great scientist.

I am grateful to Dr. Lee Ryker for assuming the major professorship following Dr. Rudinsky's death. A difficult task, Dr. Ryker filled the vacancy admirably. His comments contributed substantially to the improvement of my thesis.

I wish to extend my warmest thanks to my minor professor Dr. Everett Hansen for his unwavering confidence and financial support of my research. His invaluable friendship and personal support provided the impetus needed to complete this thesis.

In addition, I wish to thank Dr. Richard Clarke for his contribution to the formulation of my thesis proposal and Dr. Timothy Schowalter for filling in as a member of my committee following Dr. Clarke's departure. Paul Hessburg, Paul Hennon, and Phil Hamm helped with some of the field work and their efforts are greatly appreciated.

Finally, I wish to thank my parents, sister, and brothers for their continued support throughout my career.

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INSECTS ASSOCIATED WITH BLACK STAIN ROOT DISEASE OF DOUGLAS-FIR IN WESTERN OREGON

INTRODUCTION

Insects are the primary agent for dispersal and inoculation of numerous plant pathogenic organisms. Coevolutionary forces acting on the insect and the disease organism have resulted in a wide range of coupling intensities between these two dissimilar organisms. Couplings may be loose, such that the symbiosis approaches commensalism, i.e. bees and the fire blight bacterium (Agrios, 1969); or couplings may be very tight, indicative of a well adapted mutualism, i.e. blue-stain fungi and mycangial fungi and bark beetles (Barras and Perry 1975). This study explores a previously unknown relationship between a root xylem pathogen of conifers and several species of subcortical insects. The interaction of these insects and the fungus has all the signs of a symbiotic association. The association between root-inhabiting insects and black stain root disease, caused by Verticicladiella wagenerii Kendrick, has resulted in the promulgation of an insect vector hypothesis for the disease in ponderosa pine (Goheen and Cobb 1978). Only recently has V. wagenerii been discovered in Douglas-fir in the Pacific Northwest (Johnson 1976, Goheen and Hansen 1978) and very little is known of the pathogen-insect-host relations in this ecosystem. In particular, data are needed on insect species colonizing V. wagenerii-infected Douglas-fir, their basic biologies, and the population responses of these insects to human-induced modifications of forest ecosystems. This thesis is an initial exploration of insect vector-black stain root disease relationships in the Douglas-fir ecosystem.

In 1961, Wagener and Mielke reported a disease syndrome of single-leaf pinyon pine (Pinus monophylla Torr. & Frem.), ponderosa pine (P. ponderosa Laws.), and Jeffrey pine (P. jeffreyi Grev. & Balf.) characterized by a black staining of the root xylem. Upon microscopic examination, pigmented hyphae were observed within the tracheids of black-stained roots, and isolates from stained tissue consistently yielded a fungus in the hyphomycetous genus Verticicladiella. Soon

afterwards, Kendrick (1962) described Wagener and Mielke's pinyon isolate under the binomial V. wagenerii. Later reports (Smith and Graham 1975, Hunt and Morrison 1979) extended the range of V. wagenerii from California, Arizona, and Colorado to Idaho, Oregon, Washington, Utah, Nevada, and British Columbia and expanded the native coniferous hosts to include knobcone pine (P. attenuata Lemm.), sugar pine (P. lambertiana Dougl.), western white pine (P. monticola Dougl.), lodgepole pine (P. contorta Dougl.), pinyon pine (P. edulis Engelm.), western hemlock (Tsuga heterophylla (Raf.)Sarg.)[1], and mountain hemlock (T. mertensiana (Bong.)Carr.)[1]. Cobb and Platt (1967) also reported Douglas-fir (Pseudotsuga menziesii (Mirb.)Franco) succumbing to V. wagenerii.

Forest surveys reporting V. wagenerii in Douglas-fir in Oregon first appeared in 1971 (Johnson 1976). Goheen and Hansen (1978) surveyed plantations in Oregon and Washington and concluded that V. wagenerii was widely distributed throughout the western coast range mountains and the west side of the Cascade mountains. Hansen (1978) concluded that V. wagenerii-diseased trees were more commonly associated with areas adjacent to roads than with areas located at a distance from roads.

A number of publications presented histological observations on the colonization and within-tree spread of V. wagenerii and some data were available on host response to infection by this organism. Pathogenicity tests indicated that V. wagenerii penetrated intact, presumably uninjured roots or rootlets and incited disease (Wagener and Mielke 1961, Cobb and Platt 1967, Goheen 1976). Hyphae spread through the xylem of infected trees, elongating from tracheid to tracheid via bordered pit pairs (Wagener and Mielke 1961, Smith 1967, Landis and Helburg 1976). Typically, hyphae colonized the root system and root collar but sometimes also spread into the lower bole of an infected tree (Wagener and Mielke 1961, Goheen 1976). Severely infected ponderosa pine seedlings developed moisture stress, and their rates of photosynthesis and transpiration decreased (Helms et al. 1971).

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[1]D. Goheen, USFS, Region 6, Personal Communication.

Goheen (1976) examined the black stain syndrome and the epidemiology of V. wageneriі in ponderosa pine. He observed that trees adjacent to infected trees became infected via root contacts and root grafts, as observed by Wagener and Mielke (1961) for pinyon pine. Furthermore, some infections appeared to result from hyphal growth through the soil from diseased roots to adjacent healthy roots; fine rootlets were the likely infection courts in these circumstances (Goheen 1976). Hicks (1978) demonstrated mycelial growth of V. wageneriі from inoculum blocks through untreated forest soil. Also, Hicks et al. (1980) isolated V. wageneriі from soil samples taken up to 4-6 cm away from infected roots. The pattern of local spread of V. wageneriі within pine forests also supported this evidence.

Pine stands with V. wageneriі-infected trees developed characteristic pockets of mortality (Wagener and Mielke 1961, Goheen 1976). Typically, these pockets appeared as areas of standing dead trees with a perimeter of infected trees in various stages of decline. Newly infected trees were located at the outer edge of the pocket. To date, Goheen's (1976) first hypothesis predicting spread and intensification of V. wageneriі via root contact and hyphal growth through the soil has accounted for these empirical observations (Hicks 1978, Hicks et al. 1980). However, the initiation of new V. wageneriі infection foci was not explained by the hypothesis of local spread and intensification and, as late as 1975, no evidence was available to account for this process (Smith and Graham 1975). Gohenn's (1976) research in the ponderosa pine-black stain system resulted in a second hypothesis, which involved subcortical insects and V. wageneriі in an insect vector-pathogen relationship (Goheen and Cobb 1978).

Goheen and Cobb (1978) reported V. wageneriі sporulating "in situ". They observed conidiophores of V. wageneriі in the galleries of root-inhabiting insects, particularly the root scolytid, Hylastes macer LeC. Goheen (1976) discovered Ceratocystis perithecia in galleries of H. macer and determined that these perithecia were the sexual fruiting stage of V. wageneriі. From this finding, Goheen and Cobb (1978) described Ceratocystis wageneri Goheen and Cobb.

A number of other Ceratocystis pathogens of woody hosts are known to have insect vectors (Collins, 1935, Parker et al. 1941, McMullen et al. 1955, Jewell 1956, Hussain 1968, Moller and DeVay 1968, and Hinds 1972) or suspected vectors (Molnar 1965). Mechanisms of fungal infestation of all vectors were essentially similar; ascospores released from perithecia and spores on conidiophores are borne in a gelatinous matrix which readily adheres to a surface upon contact. Characteristically, these spore-producing structures are formed in the subcortical environment within insect galleries and are not generally available for wind or water-splash dispersal. Insect activity within galleries results in contact and infestation with spores of these fungi. Spore-infested beetles inoculate healthy host trees at wounds or small branches during feeding, thereby completing the transmission cycle. Barras and Perry (1975) have provided an annotated bibliography of publications dealing with Ceratocystis fungi and their association with insects.

Goheen and Cobb (1978) predicted that insect vectors would be implicated in the initiation of new V. wageneriі infection foci. H. macer was suggested as a vector in the ponderosa pine-black stain disease system (Goheen and Cobb 1978). Given these developments, it was hypothesized that within the Douglas-fir-black stain disease system exists an insect vector-V. wageneriі association providing for pathogen dispersal and introduction into susceptible hosts.

Leach (1940) provided four postulates necessary to confirm an insect vector-pathogen relationship.

1) A close, although not necessarily constant, association of the insect with diseased plants must be demonstrated;
2) It must be demonstrated that the insect also regularly visits healthy plants under conditions suitable for transmission of the disease;
3) The presence of the pathogen or virus in or on the insect in nature or following visitation of a diseased plant must be demonstrated;
4) The disease must be produced experimentally by insect visitation under controlled conditions with adequate checks.

Research Objectives

Three goals were developed to evaluate Leach's (1940) first postulate for the hypothesized Douglas-fir-*V. wageneriі*-insect system. These thesis goals were:

1) Identify, in the guild of subcortical insects associated with dead and dying Douglas-fir, those species which are also present in the roots, root collar, and lower stem of *V. wageneriі*-infected trees;
2) Describe the seasonal development and colonization of these insects within various stages of diseased trees;
3) Assess the viability of *V. wageneriі* and the occurrence of conidial structures in various stages of pathogen colonization in diseased trees, and relate disease epidemiology to insect colonization.

Establishment of a significant overlap between insect populations and viable *V. wageneriі* within diseased trees was considered critical to support an insect vector hypothesis in this system.

MATERIALS AND METHODS

Three widely separated western Oregon Douglas-fir plantations with V. wagenerii-induced mortality were selected for study: Ball Bearing Hill (T3SR5W-T2SR6W; Yamhill Co.), Velvet Creek (T17SR9W; Lane Co.), and Alsea (T14SR7W; Benton Co.). In 1979, plantations were 15 years old at Ball Bearing, 24 years old at Velvet Creek, and 14 years old at Alsea. Diseased trees at Alsea included "volunteer" trees approximately 10 years of age. In August 1979, each site was visually surveyed for diseased trees. Each diseased tree was numbered and coded with different colored flagging based on crown color and terminal growth characteristics. At that time, 335 diseased trees were grouped into four symptom classes based on crown foliage--normal green (NG--180 trees), yellow (Y--43 trees), red (R--26 trees), and defoliated (NN--86 trees). The normal green group was subdivided into two additional symptom classes based on a ratio of leader growth (T_{gr}), where:

$$T_{gr} = \frac{\text{leader growth (in cm.) this year}}{\text{leader growth (in cm.) of the single longest module of the previous five year's growth}}$$

Infected trees with obvious symptoms (NG-S--101 trees) had T_{gr} ratios less than 0.5 while infected non-symptomatic trees and trees with symptoms not including leader reduction (NG-NS--79 trees), such as resinous lesions on the lower bole or a single branch with conspicuous growth reduction, had T_{gr} ratios greater than 0.5. Ten trees were selected at each site within the plantation, at least 10 meters from areas of mortality, as a control class (NG-C). An experimental design was employed to determine the association between symptom expression, as defined above, and the numbers of each insect species colonizing the roots, root collar, and lower stem of V. wagenerii-infected trees. The design permitted testing the hypothesis that mean insect density was significantly different between diseased and healthy trees.

The trees were assigned numbers from a random numbers table independently at each site and a strip-plot variant of the split-plot design (Cochran and Cox 1957) was applied at each site. The first

factor in the analysis consisted of six treatments, i.e., the five symptom classes and the control class. Each site was divided into 4 pockets of infected trees with each pocket free from simultaneous overlap. Each tree within each pocket of diseased trees and within each treatment was ordered by (random) number and a single tree was selected for excavation from each treatment. A single strip of treatments, one tree from each symptom class, at each site and within one pocket of infected trees selected at random, was sampled once during one of four sample dates (blocks), August-September, 1979; November-December, 1979; March-April, 1980; and June-July, 1980. Site was a second factor in the experimental design. Only unwounded trees were chosen, as wounding of diseased trees had been shown to influence insect aggregation (Goheen, 1976). Trees appearing long dead, with exfoliating bark, broken branch tips, or fruiting structures of Polyporus spp. were not selected. All together, 18 trees were excavated on each sample date for a total of 72 trees over the entire year. The strip-plot design was employed primarily to eliminate any possibility of interaction between adjacent trees removed at different sample dates, as might have occurred using a completely randomized design.

Data on certain attributes of sample trees were gathered at the time of excavation. Yearly growth of the leader (i.e. a module) was measured for the last ten years after the August-September sample data indicated that measurements of the last five years growth were too few. Two sets of four midcrown branches, taken from adjacent whorls for each tree excavated, were removed and the number of years of foliage retained was estimated. The criterion for including a single year was whether the branch module for that year possessed 75% of its original complement of needles. Branches removed from different trees at a single site were of the same age.

Sample trees were excavated by hand and all roots were removed to approximately 0.3 cm xylem diameter; only a few roots were irretrievable due to their location beneath old growth stumps, rocks, or non-sample trees. Roots were identified by their unique juncture with the root collar of the sample tree.

Bark was removed from the roots and lower one meter of the stem by hand in the laboratory. All subcortical insects were collected from each root and preserved in ethanol in separate vials. Conidiophores that resembled V. wagenerii under a dissecting microscope (10X) were placed on agar slants of a selective media (Hicks et al. 1980) and labelled to identify the specific tree and root.

Xylem circumference of each root at the root collar juncture, circumference of root xylem with black stain at the root collar juncture, annual rings with black stain, and total root length were recorded. Stem xylem circumference at soil level, stem xylem circumference with black stain at ground level, and annual rings with black stain were also measured.

After the bark of each tree was removed, six isolations were made from woodchips taken from each tree, three from the lower stem and root collar and three from the roots. The chips were placed on agar slants of the selective media and incubated at 15°C. Conidiophores formed in culture by promising isolates were compared with Kendrick's (1962) description of V. wagenerii and confirmation was based on four criteria: (1) mononematous conidiophore; (2) sympodial spore formation; (3) lack of rhizoids; (4) some conidiophores with five or more primary metulae. These criteria were believed to successfully separate V. wagenerii from other species of Verticicladiella presented by Kendrick (1962). No pathogenicity tests were performed.

Insects found associated with diseased trees were identified using Blackman (1941), Hopkins (1911), Deyrup (1978), Condrashoff (1966), and Leckander (1968). The head capsule width of larvae was measured using an American Optical filar micrometer. Instars were assigned from head capsule widths by comparing measurements with those published (Condrashoff 1966, Zethner-Møller and Rudinsky 1967). Stem-inhabiting bark beetles were only noted because their association with Douglas-fir infected with V. wagenerii had been reported earlier (Goheen and Hansen 1978).

Data were analyzed by analysis of variance (ANOVA) utilizing both original values and $Log_{10}(X+1)$ transformed values. The $Log_{10}(X+1)$ transformation was used for these analyses because the mean and variance are often highly correlated (Little and Hills 1978). The split-plot design fails to partition the variance among the individual treatments and therefore the correlation between the mean and the variance could not be tested. However, considering the data as random samples the two statistics were found to be highly correlated. For this reason, the $Log_{10}(X+1)$ transformation was employed in an attempt to remove or reduce the effect of this correlation from the data analysis.

RESULTS

Effects of *Verticicladiella wagenerii* Infection on Attributes of the Host, Douglas-fir

Characteristic symptoms in Douglas-fir infected with *V. wagenerii* included reduced leader and branch growth, chlorosis, reduced needle size, reduced needle retention, resinous lesions on the lower stem, and, on occasion, a distress cone crop. Reduced leader proved to be the most sensitive indicator of colonization. In most instances, severe symptoms such as abrupt and substantial leader growth reduction were observed between two adjacent leader modules of red trees excavated at the three samples sites (Figure 1). Trees usually died in two seasons or occasionally a single season following severe leader reduction. Only a few trees survived three or more seasons. Decline of diseased trees averaged four years at Velvet Creek and three years at Ball Bearing and Alsea (Figure 2). Needle losses of the years of foliage retained for eight midcrown branches, excluding the defoliated treatment, were significantly greater (ANOVA, $\alpha < 0.01$) in diseased trees than in control trees (Table 1). Also, all symptom classes except symptomatic trees with green foliage and trees with yellow foliage were significantly different from one another. The number of years of foliage retained was significantly decreased ($\alpha < 0.01$) prior to the onset of substantial leader reduction. Non-symptomatic green trees had 82%, green symptomatic trees had 61%, and yellow trees had 54% of the number of years of foliage retained by the control treatment. Trees classified as red in August 1979 rapidly lost their needles and by December were entirely defoliated.

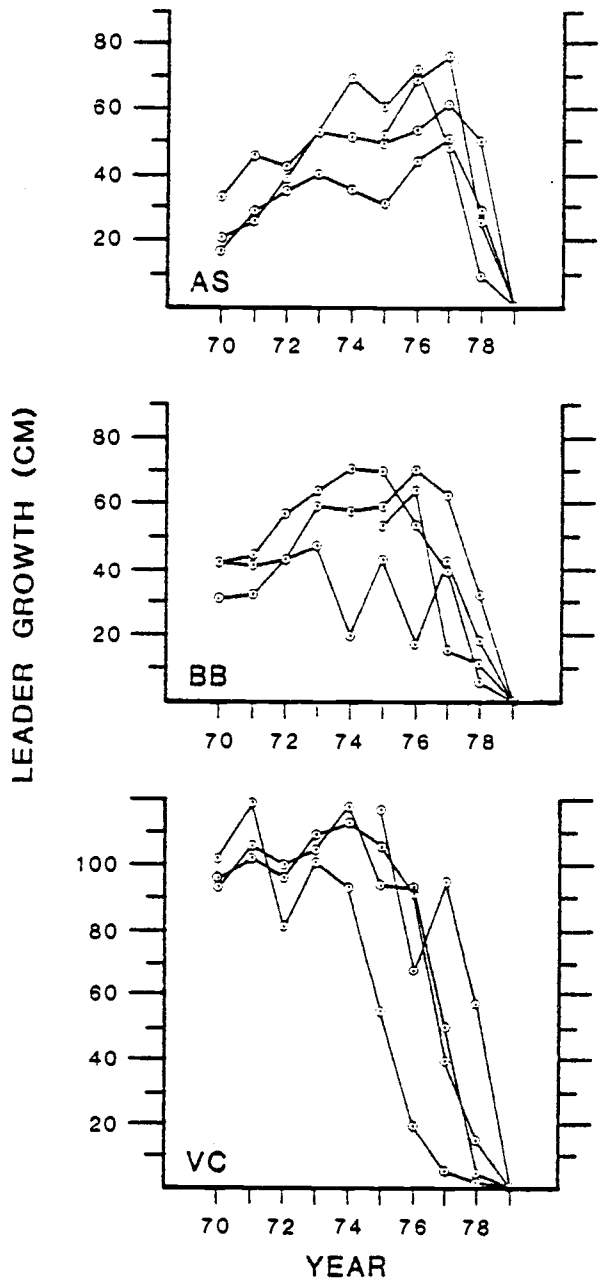

Figure 1. Annual leader growth (cm) of four Douglas-fir trees excavated at each site which were infected with Verticicladiella wagenerii and which died during 1979. AS=Alsea; BB=Ball Bearing; VC=Velvet Creek.

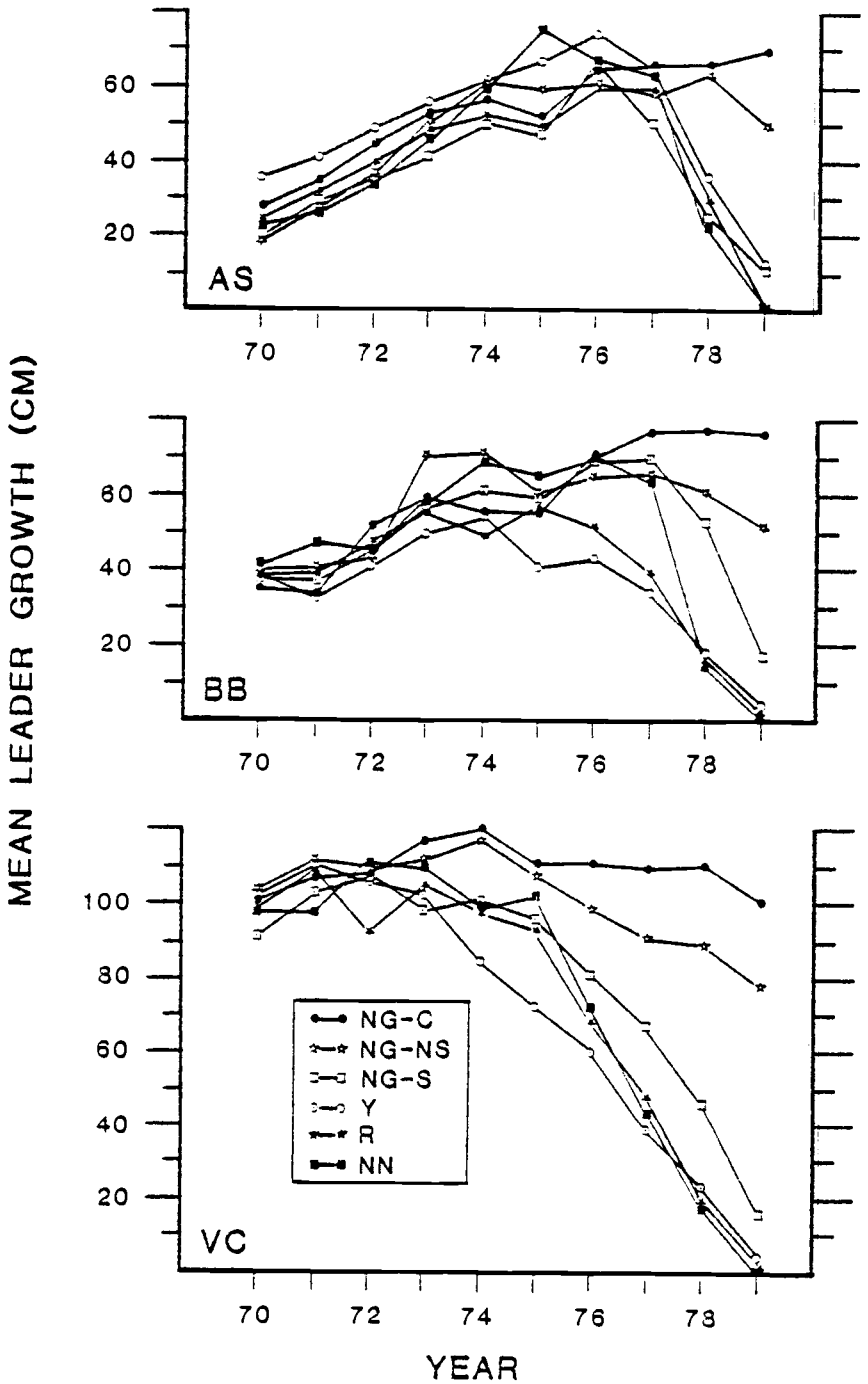

Figure 2. Annual mean leader growth of Douglas fir uninfected and showing degrees of crown symptoms resulting from infection with Verticicladiella wagenerii. AS=Alsea; BB=Ball Bearing; VC=Velvet Creek. NG-C=uninfected control; NG-NS=infected, no symptoms; NG-S=infected, short leader; Y=infected, yellow foliage; R=infected, red foliage; NN=infected, defoliated.

Table 1. Foliage retention (years) for Douglas-fir trees uninfected compared with those showing degrees of crown symptoms resulting from infection by *Verticicladiella wagenerii*[+].

Condition of Tree	Mean[1]	95% Confidence Interval
Uninfected, control (NG-C)	4.39[a*]	4.00-4.78
Infected, no symptoms (NG-NS)	3.59[b]	3.20-3.98
Infected, short leader (NG-S)	2.68[c]	2.29-3.07
Infected, yellow foliate (Y)	2.35[c]	1.96-2.74
Infected, red foliate (R)	0.14[d]	0.00-0.53

[+] based on a sample of eight midcrown branches per tree.

[1] LSC (α=0.05)=0.5146.

[*] means followed by different letters are significantly different at α=0.05.

Verticicladiella wagenerii in Douglas-fir

Root and lower stem circumference with black-stained xylem abruptly increased from $\leq$ 24% for non-symptomatic green trees to $\geq$ 63% for symptomatic diseased trees (Table 2). Red and defoliated trees were black-stained over nearly their entire xylem circumference. Linear regressions between the proportion of woody root circumference with black stain or proportion of lower stem xylem at ground level with black stain and terminal growth ratios (T_{gr}) gave slopes significantly different from zero ($\alpha < 0.01$). Using all treatments except the defoliated trees, the relationships were described by the equations:

$$T_{gr} = 0.81 - 0.87X_1 \qquad (1)$$

and

$$T_{gr} = 0.84 - 0.92X_2 \qquad (2)$$

where X_1 = proportion of stem xylem circumference with black stain and X_2 = proportion of root xylem circumference with black stain (Figure 3). The abrupt switch from non-symptomatic green to symptomatic green within one year resulted in a paucity of points in the middle region of these two regressions. However, the high correlation coefficients ($R^2 = 0.77$ for (1) and $R^2 = 0.78$ for (2) indicate a significant linear component to the relationships. Fungal colonization, as measured by proportion xylem circumference wtih black stain, had probably continued to increase after leaders ceased growing; therefore, sampling over the entire year probably contributed to the variation in these regressions.

The proportion of lower stem xylem circumference with black stain and proportion of root xylem circumference with black stain were highly correlated ($R^2 = 0.93$) and described by the equation:

$$X_1 = -0.01 + 1.01X_2 \qquad (3)$$

X_1 and X_2 are defined as in (1) and (2) above.

The fine root system of Douglas-fir heavily colonized by *V. wagenerii* was invariably destroyed by the pathogen; the rootlets were

Table 2. Root xylem circumference at root collar juncture with black-stained xylem, stem xylem circumference at ground level with black-stained xylem, and terminal shoot growth ratios of sample trees.

Measurement	Disease Symptom Category					
	NG-C*	NG-NS	NG-S	Y	R	NN
Mean proportion of root xylem circumference with black stain	0	0.24	0.64	0.68	0.86	0.92
Mean proportion of stem xylem circumference with black stain	0	0.20	0.63	0.70	0.87	0.94
Mean terminal growth ratio	0.92	0.74	0.20	0.09	0	0

* n=12 trees per treatment; NG-C=uninfected, control; NG-NS=infected, no symptoms; NG-S=infected, short leader; Y=infected, yellow foliage; R=infected, red foliage; NN=infected, defoliated.

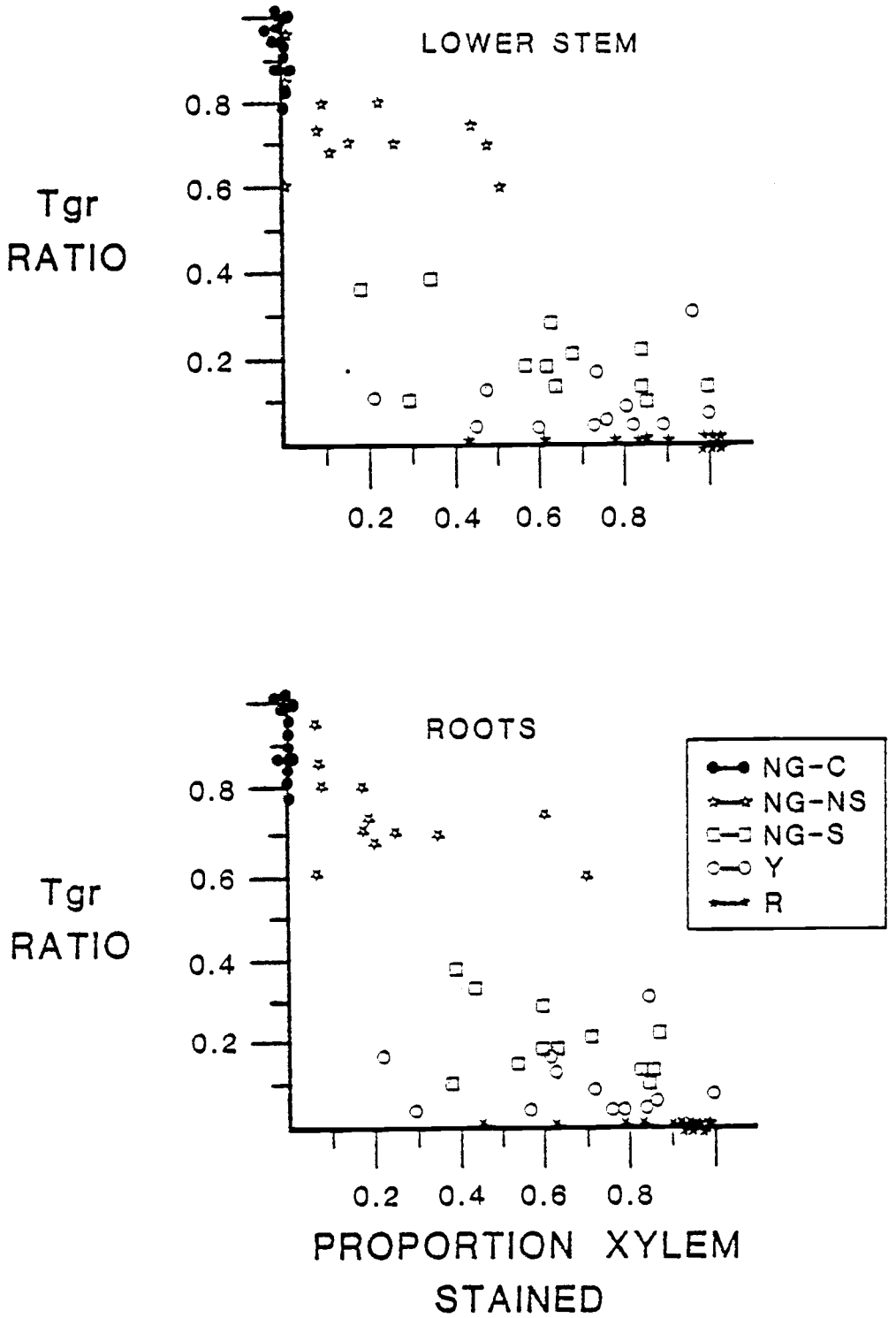

Figure 3. Correlation between proportional lower stem xylem circumference with black stain at ground level and terminal growth rate, T_{gr}, (R^2=0.77), and correlation between proportional root xylem circumference with black stain at the root juncture and terminal growth rate (R^2=0.78).

brittle and dry or resin soaked. Heavy resinousus of diseased roots was only occasionally observed in Douglas-fir. However, root junctions frequently had dead cambium and resin inpregnated bark. Lesions following cambial death were restricted to the root junction and adjacent tissue of the main root and rarely extended more than 10 cm from the root junction.

Within some lesions V. wagenerii hyphae were able to escape from xylem tracheids into the subcortical environment created when the cambium was killed and the bark had been lifted away from the xylem surface by growth of adjacent, living inner bark. Isolates of these hyphae produced viable colonies of V. wagenerii. In some roots, dense mats of hyphae were formed and conidiophores were produced. In these lesions hyphae, apparently V. wagenerii, issued from cracks and fissures in the outer bark. However, no isolations successfully recovered the fungus. Other fungi were also observed on outer bark of severely diseased and dead roots. Attempts to isolate these fungi failed because the bark of the roots had dried excessively following excavation.

V. wagenerii colonized the outer annual rings of roots and the lower stem xylem (Figure 4), preferring the early wood tracheids. The fungus typically spread from an initially infected root to the root collar and then spread through adjacent roots as described by Goheen (1976) for ponderosa pine.

Isolations from diseased trees indicated that members of all treatments contained viable V. wagenerii mycelia (Table 3). Four non-symptomatic green trees were not colonized at the lower stem and root collar, which explained the low V. wagenerii recovery rate for this group. Successful isolation of V. wagenerii decreased somewhat with increased symptom expression, but 75% of all defoliated trees contained viable mycelia. V. wagenerii was only rarely recovered from roots which were riddled with insect emergence holes. By contrast, V. wagenerii was readily recovered from roots with insect brood and from infected roots which were not colonized by insects.

Recovery of V. wagenerii from conidiophores was equivocal. The data suggest that conidiophore production was not confined to any single

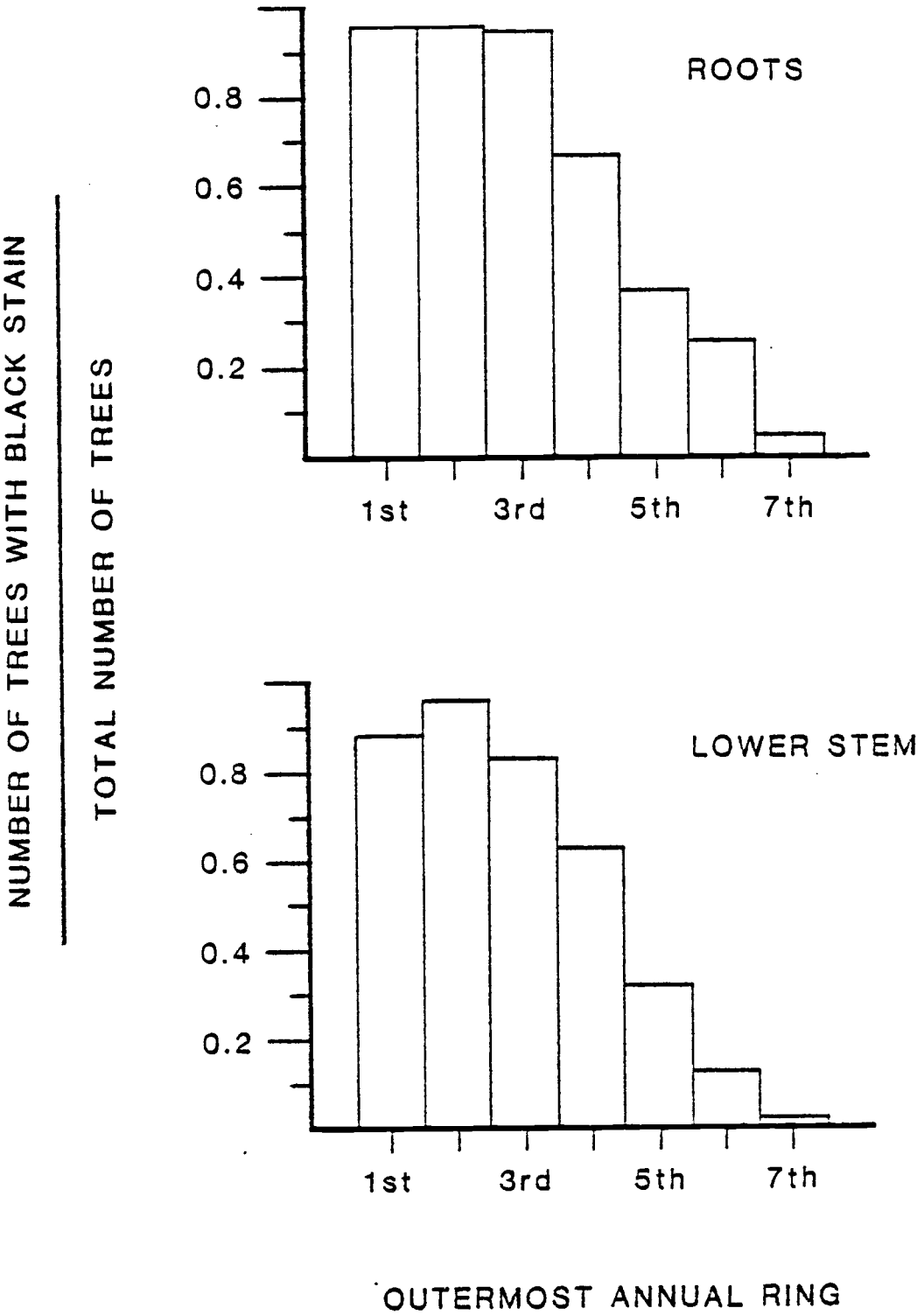

Figure 4. Proportional occurrence of black stain in xylem annual rings of roots at the root collar juncture and lower stem at ground level for sample trees.

Table 3. Frequency and percentage occurrence of viable Verticicladiella wagenerii in roots and lower stem of sample trees.

Portion of Tree	Disease Symptom Category					
	NG-C*	NG-NS	NG-S	Y	R	NN
Roots	0 (0)[1]	11 (92)	12 (100)	9 (75)	7 (58)	8 (67)
Lower stem	0 (0)	8 (67)	12 (100)	9 (75)	8 (67)	9 (75)
Either	0 (0)	12 (100)	12 (100)	10 (83)	10 (83)	9 (75)

* n=12 trees per treatment; explanation of symbols in Table 2.
1 values in () are percentages

treatment but was rather a function of the decline of individual roots (Table 4). Conidiophore production was most commonly observed in insect-infested roots and portions of roots. Within all treatments, some trees had conidiophores at sampling. The method of excavation and processing contributed to a substantial loss of information concerning conidiophore production and seasonal sporulation. Disturbance during excavation probably resulted in insects moving about in galleries and pupal cells. If conidiophores with spore drops were contacted by an insect, the conidiophores would have discharged their inoculum over the surface of the insect. Conidiophores without spore drops were not cultured. A potentially significant insect relationship to conidiophore induction will be discussed below, following a discussion of the specific insects involved.

Table 4. Successful isolation of *Verticicladiella wageneriii* from conidia from sample trees with viable mycelia[1].

Time Period	Disease Symptom Category				
	NG-NS	NG-S	Y	R	NN
August-September 1979	0/3[2]	1/3	1/3	1/2	0/1
November-December 1979	1/3	0/3	0/2	2/3	2/3
March-April 1980	0/3	2/3	0/3	1/3	0/3
June-July 1980	1/3	0/3	1/2	0/2	0/2

[1] based on at least one positive isolate from three root and three lower stem and root collar isolates per tree; explanation of symbols in Table 2.

[2] number of trees yielding successful conidial isolates/number of sample trees with viable mycelia.

Root-inhabiting Insects

Three species of beetles were consistently recovered from diseased trees, Pissodes fasciatus LeC. (Curculionidae), Steremnius carinatus (Boh.) (Curculionidae), and Hylastes nigrinus (Mann.) (Scolytidae), and constituted 97% of the insects recovered. Associated insects included beetles in the families Elateridae (3 insects), Cleridae (4 insects), Cerambycidae (27 insects), flies in the Dolichopodidae (11 insects), wasps in the Braconidae (28 insects), and other Scolytidae (2 insects). Since there were so few of these insects the analysis was confined to P. fasciatus, S. carinatus, and H. nigrinus.

Trees classified as red in August, all of which contained brood of the stem-inhabiting bark beetles Scolytus unispinosus LeC., or Pseudohylesinus nebulosus LeC., contributed 45% (1017 insects) of all S. carinatus, P. fasciatus, and H. nigrinus collected in the roots and lower stems. Non-symptomatic green trees contributed 4% (85 insects), symptomatic green trees 24% (538 insects), yellow trees 9% (196 insects), and dead trees 18% (421 insects) (Figure 5). Control trees were free of subcortical insects and their damage (Table 5). Among the yellow, red, and dead trees, insects were present or had been present, based on head capsule remains, in 100% of the sample trees (Table 6). Of diseased, symptomatic sample trees, 83-100% of the trees of any treatment were infested at the time of excavation (Table 7). In the non-symptomatic green treatment 50% (6 trees) were infested at the time of excavation; the species involved were almost exclusively S. carinatus and H. nigrinus (Table 7).

All treatments except non-symptomatic green trees had significantly (α = 0.01) more insects per tree than the control treatment, based on ANOVA of $Log_{10}(X+1)$ transformed data for total occurrence of beetle species (Table 8). The spread of V. wageneriі through the host root system, the duration of decline of the host tree, and the seasonal ovipositional episodes of the colonizing insect species suggest a rather continuous level of insect colonization and occupation of root and lower bark stem inner bark throughout the duration of decline of the host.

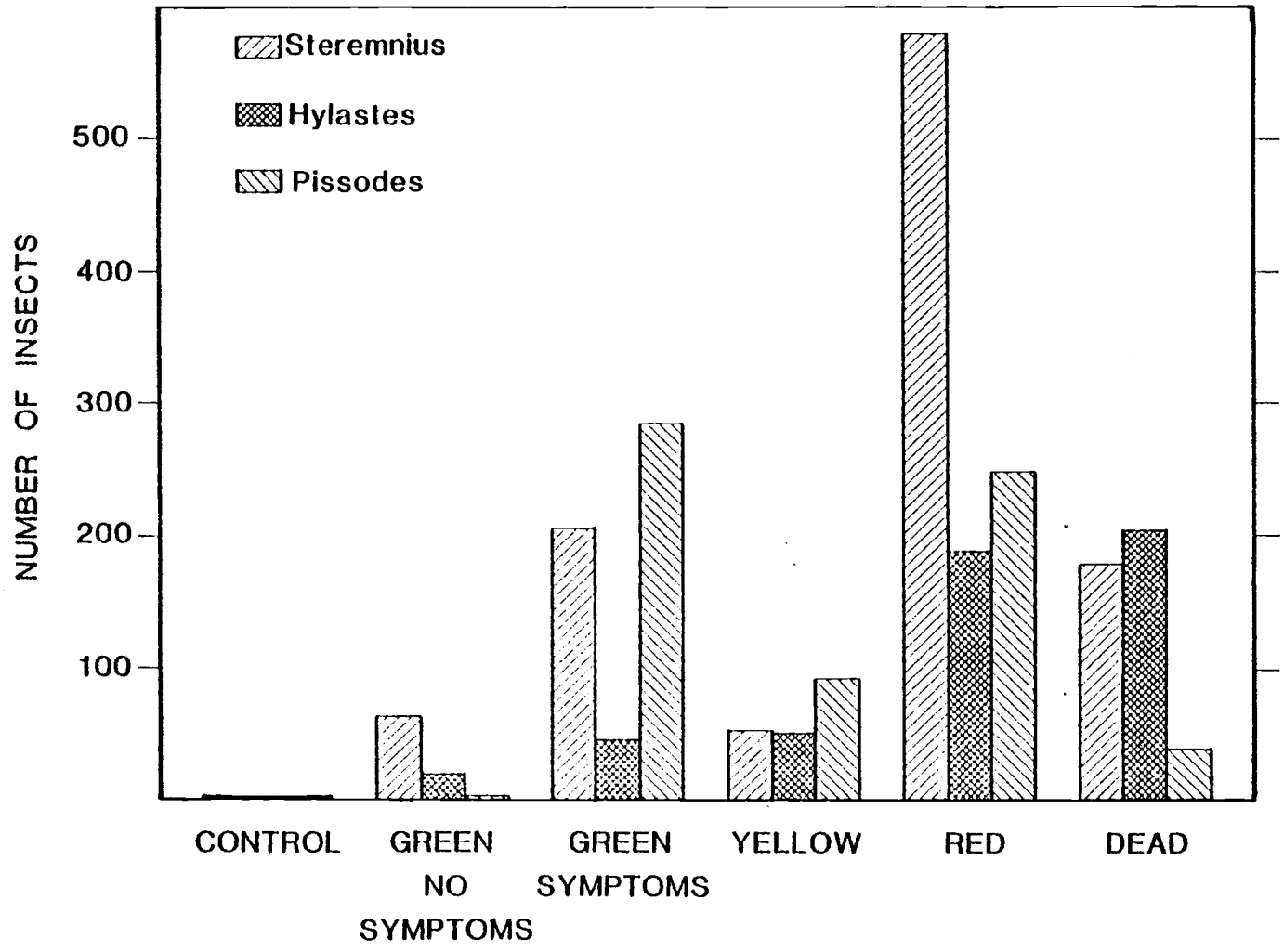

Figure 5. Number of Pissodes fasciatus, Steremnius carinatus, and Hylastes nigrinus recovered from Douglas-fir uninfected and showing degrees of crown symptoms resulting from infection with Verticicladiella wagenerii.

Table 5. Mean number of *Steremnius carinatus*, *Pissodes fasciatus*, and *Hylastes nigrinus* recovered from under bark of trees of various symptom classes.

Site	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
Alsea	0	7.8	4.8	26.0	58.0	35.5
Ball Bearing	0	0.5	19.3	11.3	39.5	21.3
Velvet Creek	0	13.0	111.3[b]	11.8	157.5	48.8

[1] symbols explained in Table 2.

[b] means are significantly different from control at α=0.05; LSD (α=0.05)=96.0; n=4 trees per treatment

Table 6. Frequency and percentage occurrence of insects or their damage in sampled trees.

Species	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
H. nigrinus	0 (0)[2]	4 (33)	7 (58)	8 (67)	11 (92)	11 (92)
P. fasciatus	0 (0)	1 (08)	7 (58)	9 (75)	12 (100)	12 (100)
S. carinatus	0 (0)	8 (67)	11 (92)	12 (100)	12 (100)	12 (100)
All insects	0 (0)	8 (67)	11 (92)	12 (100)	12 (100)	12 (100)

[1] n=12 trees per treatment; explanation of symbols in Table 2.

[2] values in () are percentages.

Table 7. Frequency and percentage occurrence of insects in sample trees.

Species	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
H. nigrinus	0 (0)[2]	4 (33)	4 (33)	5 (42)	6 (50)	8 (67)
P. fasciatus	0 (0)	1 (08)	8 (67)	9 (75)	11 (92)	6 (50)
S. carinatus	0 (0)	6 (50)	8 (67)	8 (67)	11 (92)	9 (75)
All insects	0 (0)	6 (50)	10 (83)	12 (100)	12 (100)	11 (92)

[1] n=12 trees per treatment; explanation of symbols in Table 2.

[2] values in () are percentages.

Table 8. Mean number of insects ($Log_{10}(X+1)$) per treatment per tree.

Species	Disease Symptom Category						SE	LSD α=0.05	LSD α=0.01
	NG-C[1]	NG-NS	NG-S	Y	R	NN			
All insects	0^{a*}	0.46^{a}	0.99^{b}	1.15^{b}	1.61^{b}	1.30^{b}	0.27	0.47	0.66
S. carinatus	0^{a}	0.38^{b}	0.63^{b}	0.53^{b}	1.14^{b}	0.95^{b}	0.21	0.36	0.50
P. fasciatus	0^{a}	0.05^{a}	0.64^{b}	0.74^{b}	1.02^{b}	0.33^{a}	0.23	0.41	0.58
H. nigrinus	0	0.23	0.29	0.33	0.65	0.86	0.30	NS	NS[2]

[1] n=12 trees per treatment; explanation of symbols in Table 2.

* within rows, means followed by different letters are significantly different from the control at α=0.05.

[2] because of significant interaction between treatment and site these means cannot be compared.

Pissodes fasciatus

Trees severely infected with V. wageneriі were frequently invaded by P. fasciatus weevils; percentage occurrence of P. fasciatus at sampling was highest in symptomatic green, yellow, and red trees with 67%, 75%, and 92% respectively (Table 7). The mean number of insects recovered per tree was substantially higher for symptomatic green and red trees than for yellow trees (Table 9). All red and dead trees had been damaged by P. fasciatus (Table 6). P. fasciatus was most likely to be found infesting trees dying at the time of the survey or symptomatic green and yellow trees that succumbed in the following nine months. In some instances, P. fasciatus invaded the lower stem in the same season the stem was attacked by scolytid bark beetles, but in most cases, P. fasciatus attacked the season prior to bark beetle attack on the stem. Patch killing of the stem by P. fasciatus occurred only in root collar and stem phloem tissue that was adjacent to underlying black-stained xylem.

Significantly more P. fasciatus were present per tree in symptomatic green, yellow, and red trees than in the control trees while non-symptomatic green and defoliated trees were not significantly different from the control trees (Table 8). No significant effects due to site or symptom category X site interaction were detected in the transformed data.

Development of P. fasciatus indicated one generation annually and, in this study, generations overlapped by one to three months (Figure 6). A new cohort was present in the June-July sample along with late larvae and pupae of the previous year's cohort. Head capsule measurement data were too few to calculate the precise number of instars; four instars were reported in other Pissodes species (Finnigan 1958, Stark and Wood 1964, Stevenson 1967, Silver 1968). Pupae were most abundant in the June-July and the August-September samples; adults were most abundant in the August-September sample. Adults emerged from late July through early October during 1980.

Table 9. Mean number of Pissodes fasciatus recovered from under bark of trees of various symptom classes.

Site	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
Alsea	0	0.8	2.0	13.0	39.5	0.8
Ball Bearing	0	0	14.0	6.5	6.5	7.5
Valvet Creek	0	0	55.8	3.5	16.3	1.5
$\bar{X}$	0	0.3	23.9	7.7	20.8	3.3

[1]n=4 trees per treatment per site; explanation of symbols in Table 2.

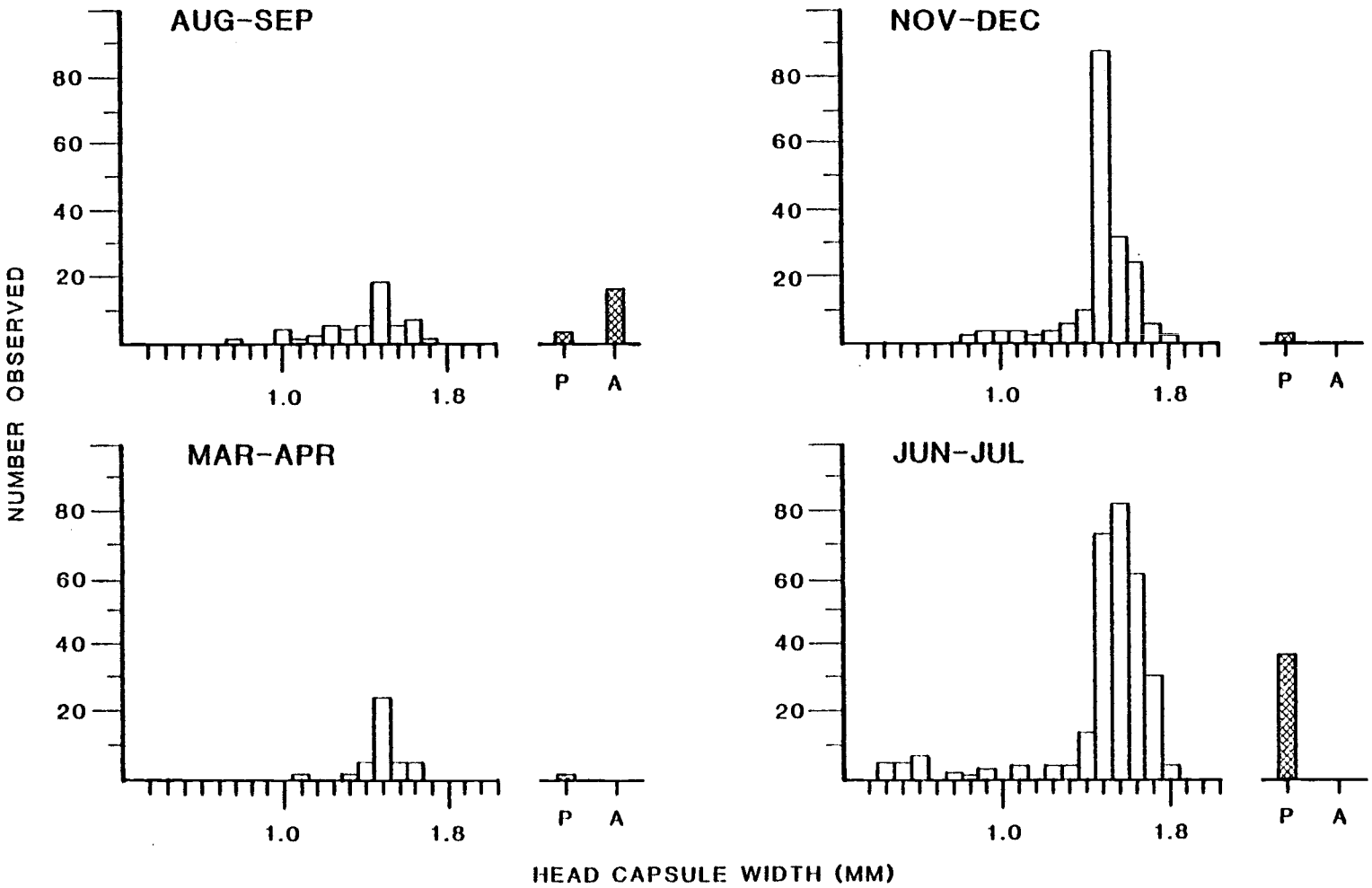

Figure 6. Larval head capsule widths and number of pupae and adults of Pissodes fasciatus recovered from sample trees in August-September 1979, November-December 1979, March-April, 1980, June-July, 1980.

P. fasciatus was observed mating from April through mid-May on the upper stems of severely diseased trees as well as the stems of adjacent, apparently healthy trees. Eggs were oviposited into lower stem phloem tissue at or above the soil line singly or in groups of from two to six as observed by Deyrup (1978). The number of larvae in roots was not significantly different from that in the lower stem and root collar (Table 10), indicating that a substantial portion of these insects mined into the roots following egg eclosion. However, P. fasciatus larvae occupied only the proximal 10-15 cm of invaded roots, supporting earlier observations by Condrashoff (1968).

P. fasciatus larvae excavated characteristic chip cocoons in the outermost xylem tissue prior to pupation. Strips of excelsior-like xylem were packed around the pupal chamber. The construction of pupal chambers disrupted xylem continuity by severing and displacing the outermost xylem, which was characteristically colonized by V. wagenerii (Figure 4). In several instances, tracheids colonized by V. wagenerii and severed during chip cocoon construction appeared to be a source of hyphae and conidiophores. When this happened, V. wagenerii gained access to adjacent insect galleries and mycelia often packed the gallery directly adjacent to the chip cocoon.

Table 10. Mean number of insects taken from roots versus lower stem and root collar per replicate[1] of sample trees, for untransformed and $Log_{10}(X+1)$ transformed data.

Species	$Log_{10}(X+1)$		F-Value	Untransformed	
	Roots	Lower Stem		Roots	Lower Stem
S. carinatus	1.51	0.50	9.47**	79.5	10.6
P. fasciatus	1.11	1.40	3.00ns	19.1	36.8
H. nigrinus	1.19	0.53	6.36*	35.5	7.0
All insects	1.90	1.57	2.74ns	133.8	54.3

[1] a replicate of sample trees included one tree from each symptom class removed during the same sample site.

* $F_{1.22df}$ (α=0.05)=4.30

** $F_{1.22df}$ (α=0.01)=7.95

Steremnius carinatus

S. carinatus was the most abundant insect collected, representing 48% of all the beetles recovered. On a relative percentage basis 27-73% of all the insects collected from the various treatments were S. carinatus (Figure 5). Only 27% of all the insects of yellow trees were S. carinatus, but 73% of all the insects in non-symptomatic green trees were S. carinatus. Occurrence of or evidence of prior occupation by S. carinatus was observed in 67% (8 trees) of the non-symptomatic green trees sampled (Table 6) with 50% (6 trees) actually infested at the time of sampling (Table 7). These data indicate that S. carinatus was most often the initial colonizer of diseased trees within established disease pockets. All red and defoliated trees had been colonized by S. carinatus.

S. carinatus was most numerous in red trees with an average of ten beetles per tree for Alsea and Ball Bearing combined and 125 beetles per tree for Velvet Creek (Table 11). Defoliated trees at Alsea and Ball Bearing and green symptomatic trees at Velvet Creek were the treatments with the second highest mean number of S. carinatus per tree.

All diseased treatments were significantly different from the control treatment (Table 8), which suggests that V. wageneriiī-diseased Douglas-fir were subjected to colonization by S. carinatus throughout the decline of the host. The site had a significant effect on the occurrence of S. carinatus. The mean numbers of beetles per tree for S. carinatus at the Velvet Creek site (0.99 beetles), based on $Log_{10}(X+1)$ transformed data, was significantly greater than the number at either the Alsea (0.36 beetles) or the Ball Bearing (0.47 beetles) sites. The mean numbers of S. carinatus from Alsea and Ball Bearing were not significantly different. Excluding the control trees, the mean number of S. carinatus was 6.5 beetles/tree at Alsea, 4.0 beetles/tree at Ball Bearing, and 43.6 beetles/tree at Velvet Creek. The trees at Velvet Creek, which produced five to ten times more S. carinatus than the other two stands, were also older (and larger) than the trees of Alsea and Ball Bearing.

Table 11. Mean number of Steremnius carinatus recovered from under bark of trees of various symptom classes.

Site	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
Alsea	0	5.0	2.5	3.5	12.0	9.5
Ball Bearing	0	0.3	2.3	1.8	8.0	7.5
Velvet Creek	0	10.3	46.8[b]	8.0	125.3[b]	27.5
$\bar{X}$	0	5.2	17.2	4.4	48.4	14.8

[1] n=4 trees per treatment per site; explanation of symbols in Table 2.

[b] means are significantly different from the control at α=0.05; LSD (α=0.05)=43.75.

The time of year of samples significantly affected the number of S. carinatus recovered (Table 12). This apparently resulted from sampling younger, volunteer trees at Alsea during March-April and June-July samples. Also, emergence of adults may have contributed to the observed differences.

S. carinatus preferred the root portion of diseased trees over the lower stems ($\alpha < 0.01$) (Table 10). Mean S. carinatus density in roots was more than seven times greater than that of lower stems (Table 10).

Histograms of larval head capsule widths from August-September and November-December samples indicate a rather broad ovipositional period; immatures were observed in all stages of development (Figure 7). New generation larvae appeared between the June-July and November-December sample dates. In the March-April sample, the S. carinatus population included primarily fourth and fifth instar larvae. Pupae and adults were most frequent in the August-September sample, but some of these stages were also present in the March-April and the June-July samples.

The developmental period of S. carinatus was approximately one year. Eggs were deposited in phloem tissue either singly or in groups of two or three. Roots as small as 3 mm xylem diameter were suitable for complete development of this insect. Larvae mined the phloem along the grain of the root wood, often directly above black-stained xylem. Fully grown larvae typically constructed chip cocoons in pupal chambers similar to those of P. fasciatus. These appeared as shallow to deep depressions in woody tissue or were entirely concealed within the root xylem (Figure 8) with a narrow, oval passageway to the inner bark and were difficult to detect when the bark was stripped off for examination. In thin roots, the chamber disrupted nearly the entire xylem vascular system.

As described for P. fasciatus, V. wagenerii within colonized tracheids severed during chip cocoon construction sometimes issued as hyphae and produced conidiophores in S. carinatus chip cocoons. In two instances, conidiophores with viable spores yielding V. wagenerii colonies were observed to line the passageway of concealed chip cocoons containing pharate adults. Isolates obtained from the adjacent, stained xylem also yielded V. wagenerii.

Table 12. Number of *Steremnius carinatus* per sample date for untransformed and $Log_{10}(X+1)$ transformed data.

Date	Untransformed		$Log_{10}(X+1)$	
	Total[1]	Mean	Total	Mean
August-September 1979	281	15.6	11.6	0.64
November-December 1979	442	24.6	15.9	0.88
March-April 1980	245	13.6	7.6	0.42
June-July 1980	113	6.3	8.4	0.47

[1] n=18 trees per sample data; ANOVA of transformed data revealed significant block (date) effects.

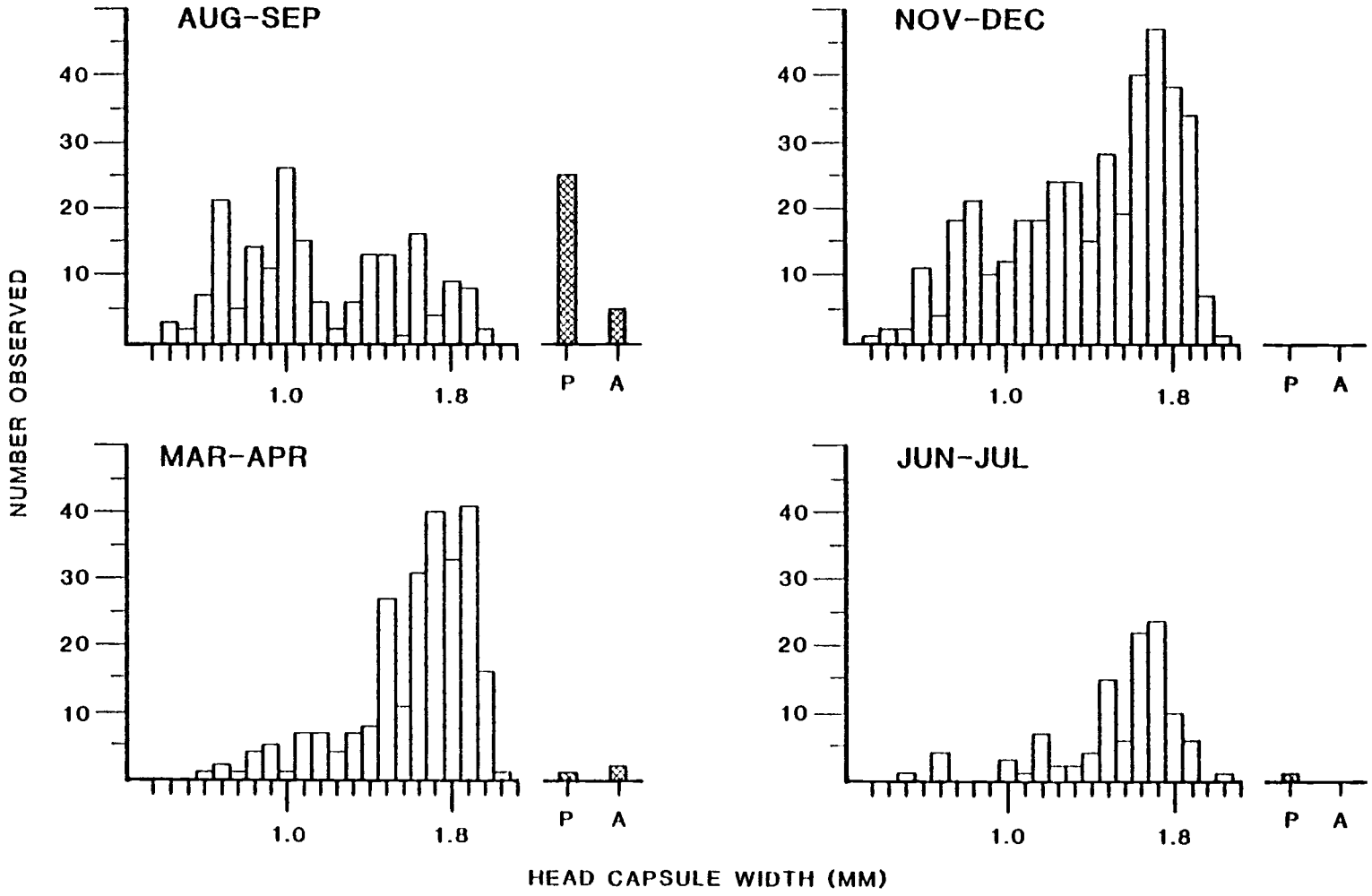

Figure 7. Larval head capsule widths and number of pupae and adults of Steremnius carinatus recovered from sample trees in August-September 1979, November-December 1979, March-April 1980, June-July 1980.

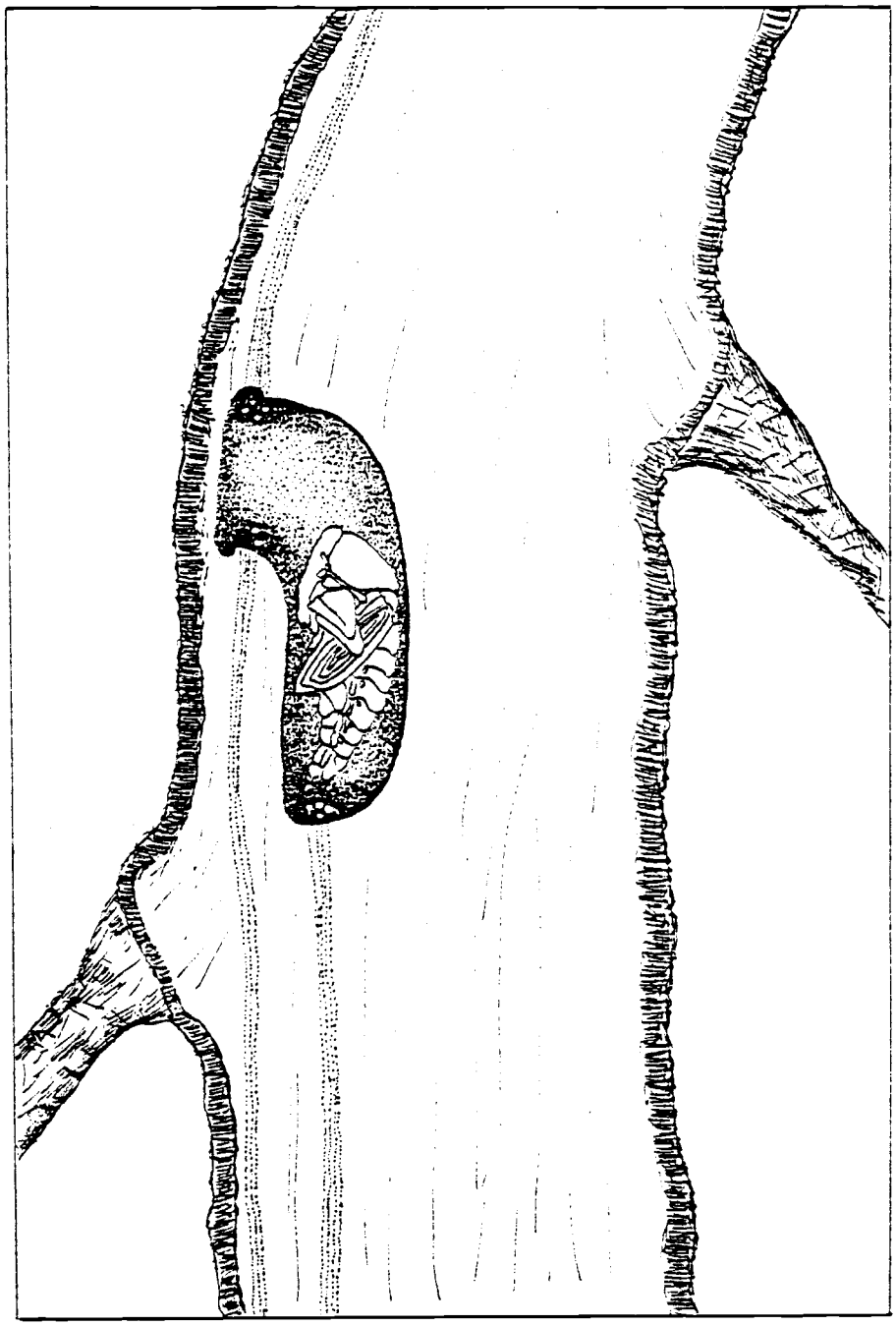

Figure 8. Steremnius carinatus pupa in chip cocoon with conidiophores of Verticicladiella wageneriii.

Hylastes nigrinus

The Douglas-fir root bark beetles, H. nigrinus, occurred most frequently in red and defoliated trees, with 50% and 67%, respectively, of sample trees infested (Table 7). Combining evidence of prior H. nigrinus occupation, 92% of red and defoliated trees had been infested by this beetle (Table 6). Numbers of H. nigrinus per tree were greatest in red and defoliated trees (Table 13). Nonetheless, H. nigrinus was collected from trees in all stages of decline; even 33% of non-symptomatic trees were infested at sampling. These data indicate that discovery and invasion of diseased trees by H. nigrinus occurred throughout the decline of the host.

Only defoliated trees on the Velvet Creek plot had significantly more H. nigrinus than non-diseased (control) trees (Table 14). Considerable variation within treatments indicated that the crown symptoms identified in this study were a poor indicator of H. nigrinus infestation.

Head capsule measurements indicated that second, third, and fourth instar larvae were present in the August-September sample and mature fifth instar larvae appeared in the March-April samples (Figure 9). A few mature larvae were still present in the June-July sample, and pupae were recovered only from the November-December and the March-April samples. Adults were equally represented throughout all sampling periods except March-April, the dispersal period for H. nigrinus (Daterman et al. 1965, Zethner-Møller and Rudinsky 1967).

H. nigrinus preferred ($\alpha = 0.05$) roots over the lower stem and root collar of diseased trees (Table 10). H. nigrinus never colonized the lower stem but was sometimes found on the underside of the root collar area, an area infrequently colonized by P. fasciatus or S. carinatus. H. nigrinus was frequently observed together with S. carinatus in roots of diseased trees.

Conidiophores of V. wageneriі were observed within H. nigrinus galleries several times. Teneral adults and numerous conidiophores were obtained from a single H. nigrinus gallery on the underside of the lower

Table 13. Mean number of Hylastes nigrinus recovered from under bark of trees of various symptom classes.

Site	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
Alsea	0	2.0	0.3	9.5	6.0	25.0
Ball Bearing	0	0.3	3.0	3.0	25.0	6.3
Velvet Creek	0	2.8	8.0	0.3	15.8	19.8
$\bar{X}$	0	1.7	3.8	4.3	15.6	17.0

[1] n=4 trees per treatment per site; explanation of symbols in Table 2.

Table 14. $Log_{10}(X+1)$ mean number of Hylastes nigrinus recovered from under bark of trees of various symptom classes.

Site	Disease Symptom Category					
	NG-C[1]	NG-NS	NG-S	Y	R	NN
Alsea	0	0.33	0.08	0.51	0.35	0.78
Ball Bearing	0	0.08	0.40	0.41	0.71	0.56
Velvet Creek	0	0.23	0.38	0.08	0.88	1.23[b]

[1] n=4 trees per treatment per site; explanation of symbols in Table 2.

[b] means significantly different from control at α=0.05; LSD (α=0.05)= 0.93.

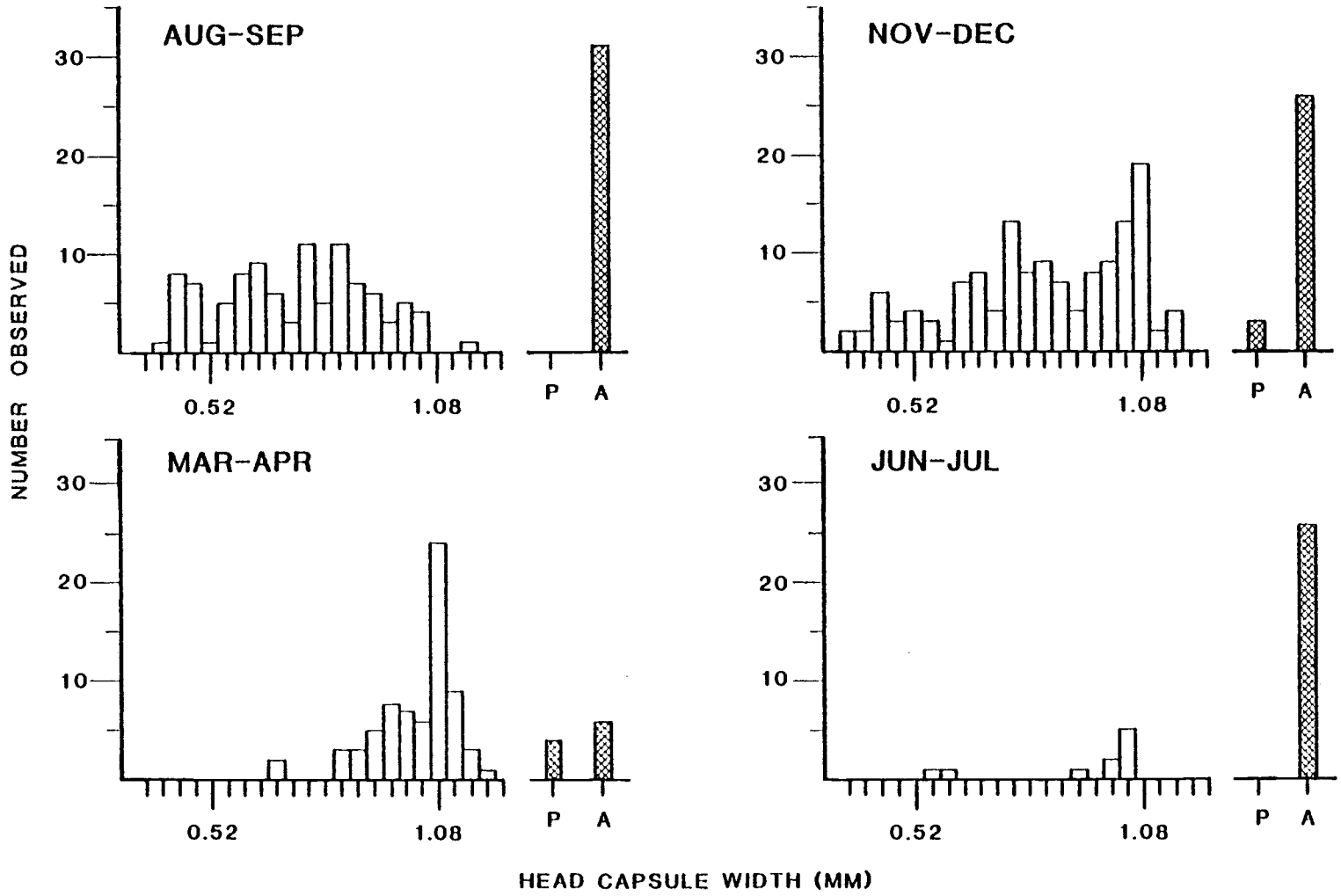

Figure 9. Larval head capsule widths and number of pupae and adults of Hylastes nigrinus recovered from sample trees in August-September 1979, November-December 1979, March-April 1980, and June-July 1980.

stem and root collar of a red tree removed in August from the Velvet Creek site. Isolates taken from spore-bearing conidiophores, from severely stained xylem directly below the gallery, and from two of eight teneral adults all yielded V. wageneriі. H. nigrinus was observed to lightly etch the xylem of roots during gallery construction, which may increase the likelihood of conidiophore production from viable hyphae in several tracheids. On occasion, V. wageneriі conidiophores were observed issuing from exposed xylem in egg and larval galleries.

Maturation feeding by H. nigrinus was reported to take place following emergence of new adults (Zethner-Møller and Rudinsky 1967). As with egg galleries, feeding galleries lightly etch the xylem of roots diseased with V. wageneriі and conidiophores were observed to issue from exposed xylem. Maturation feeding galleries of H. nigrinus frequently occurred in diseased roots but were not observed in roots that were not already diseased when excavated.

DISCUSSION

Decline of Douglas-fir infected with V. wagenerii was related to fungal colonization of the root system and lower stem. As the circumference of root and lower stem xylem infected with V. wagenerii increased, leader growth and years of foliage retained decreased proportionally. Trees with significant leader reduction had more than 60% of their entire root xylem circumference colonized by V. wagenerii and had lost more than 40% of the years of foliage retained by control trees. At this stage, most of the fine root system had been destroyed. As expressed by leader reduction, the host declines within one to four years, although the rate of decline appears to be influenced by host age. Trees 24 years of age died within four years following symptom expression while 14-15 year old trees died within three years following symptom expression.

Douglas-fir roots being colonized by V. wagenerii responded with weak resinosus associated with root branch junctions. At these locations, inner and outer bark often became resin soaked. Rudinsky (1966) and Rudinsky and Zethner-Møller (1967) demonstrated that H. nigrinus respond to a Douglas-fir oleoresin-ethanol solution and an α-pinene-ethanol solution. Pitfall traps baited with 2% racemic α-pinene in 95% ethanol capture both H. nigrinus and S. carinatus while traps without this solution catch few, if any, of these insects (Witcosky, unpublished data). Rudinsky and Zethner-Møller (1967) have also demonstrated that H. nigrinus can locate and colonize buried root sections at a distance from any root source. S. carinatus is attracted to freshly cut stem sections of Douglas-fir (Condrashoff 1968). In the present study, both of these species discovered and colonized roots well away from the root junction with the root collar, apparently by digging directly through the soil. S. carinatus, H. nigrinus, and P. fasciatus may discover V. wagenerii-infected roots and lower stem by orientation to volatile resin components associated with root branch and lower stem resinosus or perhaps other volatile substances associated with host infection.

Colonization by insects contributed to the decline and death of the tree by undermining host resistance mechanisms, induced by V. wagenerii infection, in two important ways. Adult and larval feeding damaged roots, and further, oviposition and gallery construction introduced staining fungi, including Graphium spp. and Ceratocystis spp. (Witcosky, unpublished obervations) and possibly additional V. wagenerii inoculum. Also, the insect wounds provided infection courts for soil-borne decay fungi. These insect-induced stresses apparently began prior to the onset of conspicuous crown symptoms and continued throughout the decline of the host.

Insect colonization of diseased root systems proceeded sequentially as V. wagenerii spread through the root system, heavily infected roots became suitable for oviposition. This colonization process appears to have operated at the level of individual roots rather than the root system in general, indicating that mass attack rarely takes place. V. wagenerii infection of a Douglas-fir tree, within a patch of diseased trees, results in continued invasion of the host by root-phloem consuming insects; host resistance is overcome by this pathogen-insect interaction.

H. nigrinus and S. carinatus occurred in some trees in all the stages of infection examined in this study, but S. carinatus weevils were most frequent in red trees and H. nigrinus beetles were most frequent in recently dead trees. P. fasciatus colonization appears to be associated with the death of diseased trees. Heavy infestations by this insect girdle the root collar and lower stem. Populations are most dense in red trees prior to the July-October emergence of adults; and the next most dense populations are found in symptomatic trees that succumb during the following year.

Many beetles are the vectors of other Ceratocystis pathogens (Collins 1935, Parker et al. 1941, Jewell 1956, Hussain 1968, Hinds 1972). V. wagenerii inoculum is present as viable mycelia throughout the decline of diseased Douglas-fir and is generally available as spores in insect galleries and pupal chambers. In fact, conidiophores with viable spores have been recovered from S. carinatus, P. fasciatus, and

H. nigrinus galleries containing adults. In this study, some H. nigrinus became infested with V. wageneriiinoculum within brood galleries. Thus, all three species of beetles are closely and nearly continuously associated with the disease agent in host trees and are occasionally exposed to viable inoculum prior to emergence.

It remains to be demonstrated that field collected insects carry inoculum of V. wageneriior that insects actually inoculate susceptible hosts. Assuming that insects carry viable inoculum, four possible mechanisms of inoculation are proposed:

1) Feeding or oviposition on stressed trees--Trees stressed by road construction or infected with root-rot pathogens such as Phellinus weirii (Murr.) Gilb. may become attractive to beetles which introduce V. wageneriii.

2) Maturation feeding on healthy trees--Maturation feeding has been reported for H. nigrinus and Hylastes beetles (Zethner-Møller and Rudinsky 1967) and is an important mechanism for transmission of Dutch elm disease (C. ulmi (Buisman) C. Moreau) by Scolytus multistriatus (Marsham) (Parker et al. 1941). Although no healthy roots were observed with maturation feeding galleries in this study, Rudinsky and Zethner-Møller (1967) have observed H. nigrinus galleries and root damage on apparently healthy Douglas-fir trees both inside and outside groups of windthrown trees killed by Dendroctonus pseudotsugae Hopk. S. carinatus is reported to damage planted Douglas-fir seedlings upon emergence from brood root systems of stumps following clear cutting and reforestation (Condrashoff 1968). Pissodes spp. have been reported to feed on healthy host trees following emergence of adults from brood material (Finnegan 1958, Dixon and Houseweart 1978).

3) Wounding during the host selection process--Reports indicate that bark beetles, including S. multistriatus and D. ponderosae Hopk., initiate gallery construction only following an episode of tasting of host bark (Norris and Baker 1967, Baker and Norris 1968, Gilbert and Norris 1968, Hynum and Berryman 1980).

Beetles digging through the soil may encounter, taste, and inoculate a healthy root adjacent to a diseased root or wound and inoculate roots while digging to a diseased root.

4) Inoculation associated with overwintering microhabitat--Beetles seeking overwintering sites may select microhabitats characteristically having a high density of fine roots. Inoculation may be passive; spores may germinate, grow, and infect fine roots adjacent to the overwintering beetle. Spore germination in soil was suggested by Hicks (1978).

Very little is known about feeding and overwintering processes in these insects, particularly as they relate to root disease patches or a root pathogen like V. wageneri. Our understanding of this system will be greatly enhanced by the elucidation of feeding and overwintering activities of these three beetles in V. wageneri-infected pockets of Douglas-fir.

CONCLUSIONS

Viable Verticicladiella wagenerii occur as hyphae or conidia simultaneously with insect infestation throughout the period of decline of V. wagenerii-infected Douglas-fir. Infected trees typically decline and die within two to four seasons after symptoms appear. Volatiles released by Douglas-fir in response to infection and volatiles released by host resinosus associated with root branch junctions are suspected to be cues exploited by insects dispersing to new hosts. The principal insects infesting diseased root systems and lower stems of Douglas-fir in western Oregon are Hylastes nigrinus, Steremnius carinatus, and Pissodes fasciatus.

Colonization by S. carinatus or H. nigrinus was initiated along the distal portions of heavily infected roots and spread through the root system following the progress of the fungal infection. S. carinatus and H. nigrinus may successfully produce 2-4 cohorts from a single 24 year old tree and 1-3 cohorts from a 14-15 year old tree.

Attack by P. fasciatus is associated with the death of the diseased trees, which results from a girdling of the root collar and lower stem. P. fasciatus produces 1-2 cohorts in 14-24 year old Douglas-fir, depending on whether oviposition is spatially restricted, resulting in patch killng of root collar cambium, or extensive, resulting in girdling of the lower stem and death of the tree. Stem-breeding bark beetles usually invade diseased trees in the spring following extensive P. fasciatus oviposition the previous summer.

Trees appearing red in August were the most productive sources of insects, especially S. carinatus. H. nigrinus was most abundant in recently defoliated trees and red trees. P. fasciatus was most abundant in the green symptomatic trees and red trees.

Insects are closely associated with the pathogenic fungus V. wagenerii throughout the span of host (Douglas-fir) decline (2-4 years). Some insects are likely to become infested with spores of the fungus prior to emergence and therefore represent good vector candidates. However, the mechanism of inoculation of uninfected hosts remains to be identified.

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