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Dendroctonus Valens and *Hylastes Porculus* (Coleoptera: Scolytidae): Vectors of Pathogenic Fungi (Ophiostomatales) Associated With Red Pine Decline Disease.

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DENDROCTONUS VALENS AND HYLASTES PORCULUS (COLEOPTERA: SCOLYTIDAE): VECTORS OF PATHOGENIC FUNGI (OPHIOSTOMATALES) ASSOCIATED WITH RED PINE DECLINE DISEASE.

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ABSTRACT

A study was conducted to determine whether *Dendroctonus valens* and *Hylastes porculus* could vector their commonly associated fungi to red pine. Field collected adult *D. valens* transmitted *Leptographium terebrantis*, *Leptographium procerum* and *Ophiostoma ips* into 45%, 30%, and 5%, respectively, of the wounded red pine roots onto which they were caged. Field collected *H. porculus* transmitted *L. terebrantis*, *L. procerum* and *O. ips* into 55%, 40%, and 5%, respectively, of the wounded red pine roots onto which beetles were caged. None of the control roots, which were mechanically wounded only, were found to contain *O. ips*, whereas only one control root contained *L. terebrantis* and only one control root contained *L. procerum*. This work demonstrates that *D. valens* and *H. porculus* can vector their associated *Leptographium* fungi to red pine trees and that these organisms are likely involved in red pine decline disease.

Red pine decline disease (RPDD), also known as red pine pocket decline and mortality (Klepzig and Cummings-Carlson 1988), has been detected with increasing frequency at various locations throughout the Lakes States within 30–50 year old red pines (*Pinus resinosa*) (Raffa and Hall 1988, Klepzig et al. 1991). This syndrome is characterized by an expanding circular region of dead trees and peripheral trees with reduced growth rates. Root systems of declining trees exhibit high levels of mortality and infestation with vascular, staining fungi particularly *Leptographium terebrantis* Barras and Perry and *Leptographium procerum* (Kendrick) Wingfield. *Ophiostoma huntii* (Robins.—Jeff. & David.) and *Ophiostoma minus* (Hedgcock) Sydow & Sydow also were isolated from root crowns of declining trees (Klepzig, 1989). Trees farther (> 9 m away) from the region of mortality have higher growth rates, lower levels of root mortality and lower levels of infestation with stain fungi than do trees at or near the margin of mortality.

Five species of root- and lower stem-infesting insects have been associated with RPDD (Klepzig et al. 1991). Three root weevils (Coleoptera: Curculionidae), the root collar weevil, *Hylobius radicis* Buchanan, the pales weevil, *Hylobius pales* (Herbst), and the pitch eating weevil, *Pachylobius picivorus* (Ger-

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mar) were consistently associated with areas where trees had already severely declined or died. Two bark beetles (Coleoptera: Scolytidae), the red turpentine beetle, *Dendroctonus valens* LeConte, and *Hylastes porculus* Erickson, were consistently associated with the leading edge of the expansion of decline. In these areas trees were either asymptomatic or showed only slight symptoms of decline. All five of these insects consistently carry *L. terebrantis* and *L. procerum* and occasionally *Ophiostoma ips* (Rumbold) Nannf. A third bark beetle, the pine engraver, *Ips pini* (Say), consistently carries *O. ips*. The *I. pini – O. ips* complex is invariably associated with the ultimate death of declining trees, and thus is assumed to play an important role in the syndrome (Klepzig et al. 1991, Raffa & Klepzig 1995).

Klepzig et al. (1991) proposed a model to explain the etiology of RPDD. According to this model, root and lower stem-infesting insect-fungal complexes colonize trees, and subsequently reduce their resistance against the tree-killing primary stem-infesting *I. pini* and *O. ips*. This model assumes that (1) Root beetles associated with RPDD carry *L. terebrantis* and *L. procerum* in the field and vector them into susceptible hosts; (2) Vectored fungi are pathogenic when deposited in beetle-created wounds; (3) Pathogenic fungi progress through root grafts; and 4) Root infestation reduces host resistance against stem-infesting *I. pini – O. ips* complexes. Data supporting assumption (3) were described in Klepzig et al. (1991) and support is provided for assumptions (2) and (4) in Klepzig (1994). Part of assumption (1), that *D. valens* and *H. porculus* are capable of carrying certain ophiostomatoid fungi under field conditions has also been verified. Klepzig et al. (1991) determined that 73% of *D. valens* and 46% of *H. porculus* carried *L. terebrantis* in Wisconsin. Because *D. valens*, a bark beetle which typically infests the lower stem of stressed or healthy pines, and *H. porculus*, a bark beetle which typically infests the roots of stressed trees or stumps, were most closely associated with areas of advancing decline, these two species were the focus of this study. The purpose of this study was to further test assumption (1) by determining whether *D. valens* and *H. porculus* can transmit these associated fungi to red pine.

Leach (1940) proposed four rules of proof to demonstrate insect transmission of pathogens responsible for plant disease. Insects must : (1) be commonly associated with diseased plants; (2) visit susceptible plants under conditions suitable for transmission; (3) carry inoculum in the field; and 4) successfully transmit the pathogen to plants under laboratory conditions. Only a few conifer root insects have been demonstrated to vector fungi according to these criteria. Most recently, Nevill & Alexander (1992) used Leach's postulates to demonstrate the ability of *H. pales* and *Pissodes nemorensis* Germar (Coleoptera: Curculionidae) to vector *L. procerum* to white pine. Witcosky et al. (1986) used the same criteria to demonstrate that *Hylastes nigrinus* (Mannerheim) (Coleoptera: Scolytidae), *Pissodes fasciatus* LeConte (Coleoptera: Curculionidae), and *Steremnius carinatus* (Boheman) (Coleoptera: Curculionidae) could vector *L. wageneri* Harrington and Cobb to Douglas-fir (*Pseudotsuga menziesii*). We tested Leach's postulates with *H. porculus* and *D. valens* and their associated fungi, primarily *L. terebrantis*, *L. procerum* and *O. ips*. Because the biology and host preference of the root infesting *Hylastes* spp., especially *H. porculus*, remain poorly understood, a related objective of this study was to determine the propensity of these beetles to enter and tunnel within living trees.

MATERIALS AND METHODS

Vectoring of Associated Fungi to Red Pine Roots by *D. valens* and *H. porculus.* An experiment was conducted in 1990 to determine the rates of transmission of *L. terebrantis*, *L. procerum*, and *O. ips* to roots of healthy, mature red pine and to roots of stumps by field-collected *H. porculus* and *D. valens*. The study plot was located in a healthy 44-yr.-old red pine plantation in the River Valley School Forest near Arena, Iowa County, Wisconsin.

Ten stumps, from a thinning conducted approximately 10 yr. before the experiment, and 10 apparently healthy trees were selected for treatment. Stumps were determined to be grafted to the communal root system characteristic of mature red pine plantations (Stone 1974), based on previous observations (Klepzig et al. 1991) and the fresh quality of their phloem. Three major (5 to 10 cm in diameter) lateral roots were exposed per tree (or stump) and subjected to one of three treatments: One root was mechanically wounded with a 1 cm diameter cork borer, and the disk of phloem replaced cleanly on the wound. This served as the control treatment. The other two roots per tree and stump were artificially infested with adult beetles. One root per tree (or stump) was infested with one adult female and one adult male *D. valens*. The third root on each tree and stump was infested with one adult female *H. porculus*. The roots were artificially wounded by removing a 2 × 5 cm rectangular section of outer bark. After the beetles were placed on the exposed surface of the root xylem, the bark and phloem section was replaced, the entire wound area was covered with a 3 × 6 cm section of plastic window screen (1 mm^2 mesh) and the screen was stapled onto the root. All treatments were applied at least 20 cm away from the trunk along the length of the root.

All roots were reburied, marked with flags, and sampled after 1 wk. Samples of xylem and or phloem tissue surrounding the sites of artificial infestation attempts were aseptically removed, placed in plastic bags, stored on ice and transported to the laboratory. In the laboratory, all tissue samples were surface-disinfected with ethanol, cut into smaller pieces using a flame sterilized scalpel, and placed on a selective medium for isolating ophiostomatoid fungi (Hicks et al. 1980). Isolation plates were examined after 3 to 4 d. Any colonies resembling stain fungi were noted, transferred by hyphal tipping to obtain pure cultures and identified by comparison with reference cultures from the collections of E. B. Smalley (University of Wisconsin-Madison) and T. C. Harrington (Iowa State University). Tree and stump root data were combined, as there were no differences based on host condition. Data were analyzed using a χ^2 contingency table.

Colonization of Living Red Pines by *H. porculus.* Efforts to establish *H. porculus* in 20 healthy unwounded red pine roots failed. Therefore, a second study was conducted in 1989 to determine whether this insect would enter living, but biotically stressed red pines. The study site was a 26-yr.-old red pine plantation exhibiting symptoms of RPDD in the Bakken's Pond Wildlife Management Unit, Sauk County, Wisconsin. Sixteen trees which were located along the margin of decline and thus were known to have high levels of root mortality (ca. 75%) and infection (ca. 25%) with stain fungi (Klepzig et al. 1991) were selected.

One major (5–10 cm in diam.) lateral root was exposed on each tree. Field collected adult beetles were caged onto the roots, at least 20 cm away from the trunk. In one method, three adult female and two adult male *H. porculus* were placed on the root surface underneath a 5 × 7 cm rectangular section of plastic window screen (1 mm^2 mesh). The edges of this screen were then fastened to the root with staples. Otherwise, the root was not wounded. Four living and two dead trees were infested in this manner. In the second method, a 3 × 5 cm rectangular area of bark and phloem was removed from the root before the

Table 1. Frequency of isolation of *Leptographium terebrantis*, *Leptographium procerum*, and *Ophiostoma ips* from wounded roots of living red pine trees and stumps, exposed to infestation with *Dendroctonus valens* and *Hylastes porculus* adults or mechanical wounding (Control). Total $\chi^2 = 32.49$, P= 0.0001.

| | Treatment | | |
| | D. valens | H. porculus | Control |
Fungus Isolated	(n = 20)	(n = 20)	(n = 19)
None	30%	5%	90%
L. terebrantis	45%	55%	5%
L. procerum	30%	40%	5%
O. ips	5%	5%	0%

beetles were placed on the xylem, underneath the screen section. Three living and two dead trees were infested using this method. In the third method, a 2 cm diameter hole was drilled into the root, one adult female and one adult male *H. porculus*, were placed in the hole and the hole was sealed with one half of a 2 cm diameter gelatin capsule. Three such holes were drilled in each root. Three living and two dead trees were infested using this method. All roots were marked with flags and reburied. After 4 d, all roots were uncovered and caging materials were removed. The surfaces of the phloem and xylem within the roots were carefully examined for signs of beetle infestation.

RESULTS

Vectoring of Associated Fungi to Red Pine Roots by *D. valens* and *H. porculus*. Gallery construction rates by field-collected adult *D. valens* and *H. porculus* were relatively low. *Hylastes porculus* constructed galleries within 15% of the roots onto which they were caged. The average length of *H. porculus* galleries was 2.7 cm. *Dendroctonus valens* entered 25% of the roots into which they were introduced, constructing galleries that averaged 5.6 cm in length. No significant differences between roots of trees or stumps were noted in frequency of entry by *D. valens* or *H. porculus*.

Both *D. valens* and *H. porculus* introduced their naturally borne fungi into the roots of stumps and trees (Table 1). The vectoring frequencies in roots or stumps of trees did not significantly differ, so the data were pooled. *Hylastes porculus* adults vectored *L. terebrantis*, *L. procerum* and *O. ips* to 55%, 40%, and 5%, respectively, of the wounded roots upon which they were caged. Only 5% of the wounded roots artificially infested with *H. porculus* yielded no fungi in isolations. *Dendroctonus valens* adults vectored *L. terebrantis*, *L. procerum* and *O. ips* to 45%, 30%, and 5%, respectively, of the wounded roots upon which they were caged. Only 30% of the wounded roots artificially infested with *H. porculus* yielded no fungi in isolations. Only 10% of mechanically wounded control roots yielded fungi. *Leptographium terebrantis* and *L. procerum* were each isolated from one control root, however, no insect galleries were observed in these roots. The isolation of these fungi from control roots may have been due to contamination or to low levels of these fungi in the soil.

Colonization of Living Red Pines by *H. porculus*. Adult *H. porculus* entered diseased red pine roots in several cases. In all entries, beetles constructed galleries of up to 1 cm long, similar in shape to naturally occurring galleries observed previously (Klepzig 1994). However, gelatin capsules used to cage beetles onto roots became filled with resin. All beetles caged onto

Table 2. Entry rates of adult *Hylastes porculus* in: (a) wounded vs. unwounded root-diseased red pine, and (b) dead root-diseased trees vs. living root-diseased trees.

	Beetles		Roots	
	n	% beetles entering	n	% roots entered
(a)				
Root Status				
Unwounded	30	10%	6	33%
Wounded	25	20%	5	60%
(b)				
Tree Status				
Dead	24	13%	6	33%
Living	41	20%	10	40%

roots in this manner died and did not successfully enter roots. The wound method was the most effective method of introducing beetles into red pine roots (Table 2). Wounded roots were entered by *H. porculus* 60% of the time, versus 33% of the unwounded roots. Beetles exhibited no clear preference between roots of living and dead trees, entering 40% and 33% of these roots, respectively.

DISCUSSION

Both *H. porculus* and *D. valens* are capable of vectoring ophiostomatoid fungi to red pine. Moreover, these two scolytids are apparently equally efficient vectors. However, they are more efficient at vectoring the fungi most frequently isolated from adults in the field than they are at vectoring a related ophiostomatoid species, *O. ips*, commonly carried by a related beetle in the upper stems of the same host individuals (Klepzig et al. 1991).

The rates at which these beetles carry and transmit their associated fungi in the field differ. *Dendroctonus valens* has been shown to carry *L. terebrantis* 73% of the time, *L. procerum* 7% of the time, and *O. ips* 20% of the time in red pine plantations in Wisconsin (Klepzig et al. 1991). In contrast, the percentages of red pine roots to which *L. procerum*, *L. terebrantis*, and *O. ips* were transmitted by *D. valens* were 45, 30, and 5%, respectively. *Hylastes porculus* carries *L. terebrantis*, *L. procerum* and *O. ips*, 46, 13, and 3%, respectively of the time (Klepzig et al. 1991). Rates of transmission by *H. porculus* were *L. terebrantis*—55%, *L. procerum*—40% and *O. ips*—5%. Beetles might carry facultative pathogens such as *L. terebrantis* and *L. procerum* with greater frequency than they transmit them to plant hosts due to the influence of plants on insect behavior, viability of fungal propagules, environmental conditions and the manner in which the pathogen is transmitted. However, in all cases, transmission rates of *L. terebrantis*, *L. procerum* and *O. ips* to wounded roots were similar to, or higher than, the rates at which other root- and lower stem-insects transmitted ophiostomatoid fungi in previous studies. For example, Witcosky et al. (1986) found that *H. nigrinus*, *P. fasciatus*, and *S. carinatus* vectored *L. wageneri* to Douglas-fir from 1% to 52% of the time. Likewise, Nevill & Alexander (1992) reported that *H. pales* vectored *L. procerum* to white pine (*Pinus strobus*) from 25% to 80% of the time.

The vectoring of the two root fungi most commonly associated with RPDD by the two root insects that are most commonly associated with its leading edge provides additional support for the involvement of these bark beetle/fungi complexes in this disease syndrome. Although *D. valens* and *H.*

porculus were only able to transmit fungi to wounded roots, it has been demonstrated that both of these beetles, as well as closely related species, may be attracted to wounds or host volatiles associated with wounds (Rudinsky and Zethner-Moller 1967, Owen 1985, Witcosky et al. 1987, Phillips 1990, Klepzig et al. 1991, Hobson et al. 1993). These data, despite the limited sample size, do suggest a possible role for *D. valens* and *H. porculus* in RPDD. Combined with previous studies showing that *D. valens* and *H. porculus* carry *L. terebrantis* and *L. procerum* in the field; co-occur with susceptible red pines under conditions suitable for transmission; enter and reproduce in roots of stressed, living trees; and predispose trees to attack by stem-colonizing insect-fungal complexes (Klepzig et al. 1991, Klepzig 1994, Raffa and Klepzig 1995), it seems likely that an interaction of insects, associated fungi, tree stress factors, and reduced host defensive chemistry is responsible for RPDD.

ACKNOWLEDGMENTS

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