DEVELOPMENT AND EVALUATION OF TRAPPING STUDIES FOR *HYLOBIUS PALES* (HERBST) AND *PISSODES NEMORENSIS* GERMAR (COLEOPTERA: CURCULIONIDAE) IN VIRGINIA CHRISTMAS TREE PLANTATIONS

by

Christopher John Fettig

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APPROVED:

Scott M. Salom

S. M. Salom, Chairman

Jodi Gray

J. A. Gray

James E. Johnson

J. E. Johnson

Roger R. Youngman

R. R. Youngman

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Dr. Scott M. Salom, Chairman

Department of Entomology

(ABSTRACT)

The pales weevil, *Hylobius pales* (Herbst), and the deodar weevil, *Pissodes nemorensis* Germar, are regeneration pests of pine plantations in the eastern United States. Attempts to sample regeneration weevils in Virginia have met with little success. Two trap types were field tested to determine their effectiveness in sampling *H. pales* and *P. nemorensis* populations in Virginia Christmas tree plantations. Labor intensive pit traps, using natural host materials and synthetic volatiles, caught significantly more weevils than PVC pitfall traps baited with synthetic volatiles alone. No differences in trap catches were observed between stationary and rotated traps. Vegetation management had no effect on trap catch. However, it was observed that newly planted white pine seedlings (*Pinus strobus* L.) were fed upon by *H. pales* at significantly higher rates in plots not managed for competing vegetation than in herbicide treated plots. Feeding activity in mowed plots was intermediate. Trap catch did not correlate with seedling damage within or among sampling periods, or between years. The seasonal activity of both species is reviewed in detail. Mark-and-recapture techniques used to assess trap efficacy showed traps baited with pine material were most effective, irrespective of trap type. The response of *H. pales* adults to different ethanol-and-turpentine ratios in a laboratory bioassay did not vary with respect to gender or age. No gender differences in response to treatments were observed in these studies.

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DEDICATED

TO

THE LATE BYRON T. DOWELL

A CLOSE FRIEND AND FINE COLLEAGUE

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CHAPTER 1

Introduction and Literature Review

The pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae) was first identified as a pest of pine in the northeastern United States and southeastern Canada (Carter 1916, Peirson 1921). Following the 1950's, it became a major regeneration pest of pine plantations in the southern United States (Beal and McClintick 1943, Fox and Hill 1973). More recently, *H. pales* and to a lesser extent the deodar weevil, *Pissodes nemorensis* Germar, have been implicated as vectors of the pathogenic fungus, *Leptographium procerum* (Kendr.) Wing., to eastern white pine (*Pinus strobus* L.) trees (Nevill 1990, Nevill and Alexander 1992a, b). Transmission of the fungus is believed to occur through cambial feeding, eventually resulting in the development of procerum root disease (PRD) in some *P. strobus* Christmas tree plantations (Nevill and Alexander 1992a, b). Christmas tree production has become an increasingly important industry to southwest Virginia in recent years (Nichols and Torbert 1989), ranking ninth among Christmas tree producing states in the United States (Anonymous 1986). The current PRD outbreak threatens this industry with substantial economic losses, and has forced a planting conversion from *P. strobus* to other species.

THE INSECT VECTORS

Pine reproduction weevils (Coleoptera: Curculionidae) are known to be important pests of a number of *Pinus* spp. in the midwestern and eastern United States. Adults are attracted to resinous volatiles produced by dead and dying trees as a result of natural or cultural actions (Carter 1916, Peirson 1921, Lynch 1984). They are considered a regeneration pest of forest plantation, nursery, and Christmas trees, and also feed on the branches and twigs of older trees. The term "pine reproduction weevils" refers to a complex of curculionid species that feed subcortically on *Pinus* species. In Virginia, this complex most notably includes *H. pales* and *P. nemorensis* (Anderson 1980).

Hylobius pales **Herbst**

Hylobius pales was first identified as an economically damaging pest in Petersham, Massachusetts during 1914 (Peirson 1921). In the southern United States, *H. pales* damage was not observed until the 1940's following the initiation of intensive forest management practices that created ideal conditions for weevil development (Beal and McClintick 1943). The insect breeds in the stumps, root collars, and roots of stressed or recently killed coniferous species. Known hosts consist of 29 species in 11 genera, including: *Abies, Betula, Cupressus, Fraxinus, Juniperus, Larix, Picea, Pinus, Pseudotsuga, Thuja* and *Tsuga* (Lynch 1984) (Table 1). In the northeastern United States, *P. strobus* is considered the preferred host. In the southeastern United States, *P. strobus,* loblolly pine (*Pinus taeda* L.), and slash pine (*Pinus elliottii* L.) are among the three most preferred hosts (Sentell 1949, Thomas and White 1971, Walker 1974).

Table 1. Known hosts of *Hylobius pales* (Herbst).

Scientific name	Common name	Source
Abies balsamea (L.) Mill.	balsam fir	Carter 1916
Betula populifolia Marshall	gray birch	Peirson 1921
Cupressus arizonica Greene	Arizona cypress	Beal & McClintick 1943
Fraxinus americana L.	white ash	Peirson 1921
Juniperus virginiana L.	eastern red cedar	Peirson 1921
Juniperus communis L.	common juniper	Carter 1916
Larix decidua Mill.	European larch	Peirson 1921
Larix laricina (Du Rio) Koch	eastern larch	Carter 1916
Picea abies (L.) Karsten	Norway spruce	Carter 1916
Picea pungens Engelm.	Colorado blue spruce	Corneil & Wilson 1980
Picea rubens Sarg.	red spruce	Peirson 1921
Pinus banksiana Lamb.	Jack pine	Holt & Bramble 1956
Pinus cembroides Zuccar.	Mexican pine	Wells 1926
Pinus echinata Mill.	shortleaf pine	Beal & McClintick 1943
Pinus mugo Turra	mugo pine	Wells 1926
Pinus nigra Arnold	Austrian black pine	Finnegan 1959
Pinus palustris Mill.	longleaf pine	Beal & McClintick 1943
Pinus ponderosa Laws	ponderosa pine	Carter 1916
Pinus resinosa Aiton	red pine	Carter 1916
Pinus rigida Mill.	pitch pine	Harris 1841
Pinus serotina Mill.	pond pine	Ciesla & Franklin 1965
Pinus strobiformis Engelm.	southwestern pine	James 1959
Pinus strobus L.	eastern white pine	Carter 1916
Pinus sylvestris L.	Scots pine	Carter 1916
Pinus taeda L.	loblolly pine	Beal & McClintick 1943
Pinus virginiana Mill.	Virginia pine	Davis 1961
Pseudotsuga menziesii Franco	Douglas-fir	Carter 1916
Thuja occidentalis L.	northern white cedar	Peirson 1921
Tsuga canadensis (L.) Carriere	eastern hemlock	Carter 1916

Damage

Hylobius pales adults cause seedling mortality in Christmas tree plantations just as they do in newly established forest plantations (Lynch 1984). Adult weevils feed subcortically on the tender bark of seedlings, often resulting in girdling of the stem. Seedling mortality from weevil feeding has been recorded as high as 90%, with losses commonly exceeding 40% (Peirson 1921, Nord *et al.* 1982). Weevil feeding can affect the form and symmetry of seedlings, resulting in culled trees at harvest (Holt and Bramble 1956). Economic losses tend to be higher in selectively cut plantations (choose and cut farms) which provide new breeding material annually (Corneil 1981). Most damage is caused by overwintering adults that emerge the following spring to feed on newly planted seedlings.

Hylobius pales adults inflict aesthetic damage to Christmas trees by feeding on stems, branches, and buds. Feeding damage causes shoot flagging and deformity, which reduce the value and marketability of the tree (Lynch 1984). Possible indirect damage may result from *H. pales* transmitting *L. procerum* conidiospores to pine trees, resulting in the development of PRD in some *P. strobus* plantations (Nevill and Alexander 1992a, b).

Life history

Studies by Peirson (1921) and Finnegan (1959) indicate *H. pales* has only one generation or a partial generation annually in the northeastern United

States and southeastern Canada. *Hylobius pales* goes through one complete generation and a second partial overlapping generation annually in the southeastern United States (Anderson 1980), where it overwinters in both the adult and larval stages (Beal and McClintick 1943, Doggett *et al.* 1977, Anderson 1980). In western Virginia, *H. pales* adults are most active from April through July. However, the weevil does not diapause in its southern range, but merely becomes inactive at low temperatures (Anderson 1980). Clark (1975) observed oviposition to cease by late December, suggesting that a reproductive diapause may exist.

Observation of stumps in Virginia Christmas tree plantations indicate that April and May are the peak egg-laying months for *H. pales* (Anderson 1980). Following oviposition, incubation of the egg stage is reported to be 10-14 days under field conditions (Rennels and Fox 1970). Larvae feed and develop on the cambium, phloem and xylem tissues of stumps and roots. Finnegan (1959) reported that 80% of larvae develop in the roots, with the remaining 20% developing in the stump, directly below the root collar. Larvae undergo 5 or 6 instars depending on the physiological condition of the fifth instar (Finnegan 1959). Mature larvae construct chip cocoons in outer portions of the sapwood, and following a short period of inactivity, begin pupation in those cocoons. Emerging adults chew a round exit hole through the bark and move directly to the soil surface (Thatcher 1960). Eggs laid in early spring produce adults by

August through October of that year. Eggs which are laid in late spring and early summer produce larvae that overwinter and emerge as adults the following summer (Speers 1974). Salom *et al.* (1986) report the development time of *H. pales* to be 92.5 d at 21^0 C under laboratory conditions.

Control: *Forestry*

Successful control of *H. pales* in forest plantations relies on the use of both cultural and chemical-based tactics (Nord *et al.* 1982). Currently, the principle cultural approach for managing *H. pales* emphasizes timely harvests, stump sanitation, and delayed planting strategies that reduce attractiveness of the site to feeding weevils. In the South, the most common non-chemical control is delayed planting for a period of at least one year following harvest (Salom unpublished data). Weevils emerge to find the site void of a suitable food source and are thereby forced to disperse to a more suitable environment. Although this approach is biologically successful, the long duration between harvest and regeneration is often not economically feasible for intensive forest management practices. Stump removal and sanitation offer potential control by preventing the build-up of weevil populations through the destruction of breeding habitats (Lynch 1984). Insecticides are often applied to areas of heavy infestation. Often stumps are drench-sprayed with Lindane® (Gamma BHC) or Dursban® (chlorpyrifos). In the Northeast, recommendations vary considerably among states. Delayed planting, stump sanitation, and dipping seedlings in insecticides prior to planting are among the top recommendations (Salom unpublished data). Seedling dips provide inexpensive control and allow regeneration immediately following harvest.

Control: *Christmas tree plantations*

Annual shearing and harvesting practices lead to a continuous production of material attractive to weevils, making cultural control efforts less successful than in forest settings (Weidhaas 1989). In Virginia, registered insecticides include Dursban® and Lindane® as both seedling and stump sprays, Asana® (esfenvalerate) as a seedling spray only, and Imidan® (phosmet) as a top dip for seedlings (Day *et al.* 1995). Currently, less toxic biologically-based compounds are being considered for their antifeedant properties (Salom *et al.* 1994). Stump and slash sanitation are less successful means of control than chemical-based tactics. However, excessive slash should be removed from plantations when economically viable, as this material provides additional forage resources for *H. pales* adults (Nord *et al.* 1982).

Sampling techniques

Several attempts have been made to "trap out" weevil populations; however, they have not been shown to be operationally successful (Peirson 1921, Thomas and Hertel 1969). Nevertheless, the successful monitoring of weevil populations using similar methods is an essential component of any pest management program (Hunt and Raffa 1989). The inability to accurately quantify weevil population leads to generalized management prescriptions and a reliance on insecticide usage without knowledge of pest densities. By establishing an effective sampling system, we may be able to detect when serious infestations are imminent, or provide a risk rating system by correlating weevil numbers with seedling damage. This information could reduce our reliance on insecticide usage by limiting the application of these materials to sites where damaging infestation levels are predicted to occur.

Several techniques have been used to sample *H. pales* populations. Shenefelt and Benjamin (1955) found adult weevils were attracted to light traps. Flight traps have been used to sample weevils attracted to wounded pines (Hines and Heikkenen 1977). Whole tree collections and sifting duff samples have also been used with limited success (Lynch 1984). Thomas and Hertel (1977) constructed traps made of 15 X 15 cm masonite panels, painted white on the uppermost side to reflect solar radiation, and baited with lures containing ethanol and terpenes.

Studies show traps incorporating natural pine material are effective in trapping weevils. Doggett *et al.* (1977) found pit traps to be 2-4 times as effective as disk traps in North Carolina. Freshly-cut pine bolts, 7.5 to 12.5 cm in diameter and 61 cm long, were placed in a shallow pit lined with perforated plastic and covered with fresh slash. Treatment of traps with a registered insecticide eliminated the need for daily collection. *Hylobius pales* adults have been collected from split pine billets placed on bare mineral soil (Taylor and Franklin 1970, Lawrence 1975) .

Doggett *et al.* (1977) also examined traps consisting of freshly-cut, 5 cm thick discs, 12.5 to 17.5 cm in diameter. Disc traps were placed in a cavity 5 to 7.5 cm deep, 17.5 to 25 cm in diameter, and covered with fresh slash. They were found to be less effective than the pit traps. This may result from the associated decrease in the amount of cambium and phloem tissue available to feeding weevils. Anderson (1980) tested disc traps and found it necessary to coat discs with an insecticide to retain feeding weevils.

Natural host material used in various trapping methods is difficult to standardize due to variations in monoterpene compositions and release rates. Providing natural host material is also more costly and labor intensive than the use of dispensers containing synthetically-produced host volatiles. In order to overcome these drawbacks, a PVC pitfall trap baited with host volatiles was designed for sampling European pine weevil, *Hylobius abietis* L., populations in Sweden (Tilles *et al.* 1986a, b). The trap was constructed from 20 cm lengths of 11 cm. dia., 2 mm thick, grey PVC plastic drainpipes. Caps were placed on both ends and holes were drilled around the circumference of the trap, 3 cm below the top. Traps placed in the ground with entrance holes flush with the soil surface were most successful in catching weevils (Nordlander 1987). Similar traps were used for trapping *H. pales*, the pitch-eating weevil, *Pachylobius* *picivorus* (Germar), and the root collar weevil, *Hylobius radicis* Buchanan, in Wisconsin Christmas tree plantations (Rieske and Raffa 1991, 1993). They found *H. pales* were most attracted to a 5:1 ethanol-and-turpentine ratio released at 200 mg/d of ethanol and 40 mg/d of turpentine for a 1:1 volumetric ratio. Rieske and Raffa (1993) also examined PVC pitfall traps at different trap densities and found trap catch was independent of trap density. The sampling of *H. pales* populations using PVC pitfall traps was unsuccessful in Floyd County, VA during the spring of 1991. The reason for this lack of success is unknown (J. Gray, personal communication). Researchers in Great Britain also have similar problems successfully implementing this sampling technique (Wilson and Day 1995). An effective sampling technique using the PVC pitfall trapping system baited with synthetic host volatiles would be highly desirable, but does not currently exist in these regions.

Pissodes nemorensis **Germar**

The deodar weevil (=eastern pine weevil; northern pine weevil), *Pissodes nemorensis* Germar (=*Pissodes approximatus* Hopkins) (Coleoptera: Curculionidae) is a pest of cedar and pine in the eastern United States and southeastern Canada (Johnson and Lyon 1991). Britton (1918) first established a relationship between *P. nemorensis* and pine trees. This insect breeds in the trunks, branches, and shoots of stressed or recently killed coniferous species. In the northern United States, *P. nemorensis* is closely associated with red pine (*P.*

resinosa Aiton), *P. strobus*, Scots pine (*P. sylvestris* L.) , and Virginia pine (*P. virginiana* Mill.). In the southern United States, this weevil feeds primarily on *P. taeda*, shortleaf pine (*P. echinata* Mill.), and longleaf pine (*P. palustris* Mill.). It is also a pest of exotic cedars, including the deodar cedar (*Cedrus deodara* Loud.), hence its common name (Johnson and Lyon 1991) (Table 2).

Damage

Pissodes nemorensis is considered a secondary pest that rarely weakens or kills healthy trees. It causes injury during both the larval and adult stages. Adults bore holes in the bole, branches, and shoots which may cause chlorosis of small branches and twigs (Johnson and Lyon 1991). Brood maturation in shoots may kill the terminal resulting in poor symmetry and culled trees at harvest. *Pissodes nemorensis* was reported as an important pest of coniferous plantations in Pennsylvania (Holt 1956). Finnegan (1958) found *P. nemorensis* killed 9% of Austrian black pine (*Pinus nigra* Arnold) seedlings in one plantation in southern Ontario.

Life history

Oviposition occurs near the bottom of the tree and in the deepest shade, beginning in early spring and extending for a period of several weeks. Anderson (1980) observed peak oviposition from March through May in Virginia Christmas tree plantations. However, a small percentage of females

Table 2. Known hosts of *Pissodes nemorensis* Germar.

Scientific name	Common name	Source
Cedrus atlantica Man.	Atlas cedar	Johnson & Lyon 1991
Cedrus deodara (Roxb.) Loud.	deodar cedar	Johnson & Lyon 1991
Cedrus libani Loud.	cedar of Lebanon	Johnson & Lyon 1991
Picea abies L.	Norway spruce	Blatchley & Leng 1916
Picea glauca (Moench) Voss	white spruce	Smith & Sugden 1969
Picea mariana (Mill.) B.S.P.	black spruce	Smith & Sugden 1969
Picea pungens Englem.	Colorado blue spruce	Smith & Sugden 1969
Picea rubens Sarg.	red spruce	Blatchley & Leng 1916
Pinus banksiana Lamb.	jack pine	Smith & Sugden 1969
Pinus cembra L.	Japanese white pine	Hard 1962
Pinus contorta Dougl. ex Loud	lodgepole pine	Smith & Sugden 1969
Pinus densiflora Sieb. & Zuccar.	Japanese red pine	Holt 1956
Pinus echinata Mill.	shortleaf pine	Blatchley & Leng 1916
Pinus nigra Arnold	Austrian black pine	Finnegan 1958
Pinus palustrus Mill.	longleaf pine	Johnson & Lyon 1991
Pinus pungens Lamb.	mountain pine	Hopkins 1911
Pinus resinosa Ait.	red pine	Blatchley & Leng 1916
Pinus rigida Mill.	pitch pine	Blatchley & Leng 1916
Pinus strobus L.	eastern white pine	Blatchley & Leng 1916
Pinus sylvestris L.	Scots pine	York 1933
Pinus taeda L.	loblolly pine	Smith & Sugden 1969
Pinus virginiana Mill.	Virginia pine	Blatchley & Leng 1916

have an extended ovipositional period that carries into the fall season (Hard 1962). Eggs are laid either singly or in groups of 2 to 5 in the bark of stumps and standing trees weakened or recently killed (Martin 1964). Larval stages of *P. nemorensis* may be present throughout most of the year (Johnson and Lyon 1991). Larvae feed on the inner bark while tunneling between the cambium and phloem tissues. They rarely feed below the root collar, and have been found feeding on the stems and branches of standing trees, up to 2.5 m above the ground (Martin 1964). Larval development is reported to be 36 days in southern Ontario (Finnegan 1958). Anderson (1980) reported larval development time in southwest Virginia to be 41 days by stump observations from Christmas trees harvested the previous winter.

A chip cocoon is constructed and pupation occurs similar to that observed in *H. pales*. Emerging adults feed for a short period of time on the tender bark of young trees and then enter the duff to overwinter (Martin 1964). Anderson (1980) found *P. nemorensis* adults present on stumps in January and February, suggesting that like *H. pales, P. nemorensis* does not diapause in its southern range but merely becomes inactive at low temperatures. Overwintering adults emerge in spring and fly in search of suitable forage and ovipositional hosts. The female dies shortly after laying all her eggs (Finnegan 1959), and therefore adults are most commonly found prior to the peak ovipositional period.

Control & Sampling Techniques

Control measures and sampling techniques for *P. nemorensis* are similar to those used in the management of *H. pales* infestations. Both cultural and chemical-based tactics are carried out by forestry personnel. However, because of the extended ovipositional period of the female *P. nemorensis*, chemical control tactics are less effective. Chemical treatments would have to be applied in early spring and retain efficacy for several months. Therefore, cultural control methods are relied on more heavily than in *H. pales* infestations. Stump and slash sanitation play a key role in the control of *P. nemorensis*.

THE PATHOGEN

Leptographium procerum (Kendr.) Wing., the likely causal agent of procerum root disease (PRD), is an imperfect fungus (Hyphomycetes, Dematiaceae) that was first described by Kendrick (1962) as *Verticicladiella procera* (Carlson 1994). Wingfield (1985) later reclassified the organism to the genus *Leptographium*. This fungus is a pathogen of pine present in many forest ecosystems including natural forests, seed orchards, landscape plantings and particularly, Christmas tree plantations (Bertagnole *et al.* 1983, Lackner and Alexander 1984).

Impact and Symptoms

It was not until 1967 that *L. procerum* was recognized as a pathogen of pine in the eastern United States (Dochinger 1967). Procerum root disease was first observed in *P. strobus* and *P. sylvestris* plantations in the late 1970's (Lackner and Alexander 1982, 1984). Lackner and Alexander (1982) surveyed eight Virginia Christmas tree plantations and found losses to exceed 700 saleable trees (6-10 years old) at an estimated value of $5-15 per tree. Today, disease severity has increased to an annual loss of 800,000 *P. strobus* Christmas trees valued at over $6.4 million (Nevill and Alexander 1992a).

Leptographium procerum colonizes the root collar, adjacent roots, and the lower stem of *P. strobus* and *P. sylvestris.* PRD is not a root rot, and therefore no decay is associated with the colonization of wood tissues (Carlson 1994). *Leptographium procerum* colonizes axial and ray tracheids and ray parenchyma at the root collar region of the infested host (Horner 1985). Colonization by the fungus causes delayed budbreak and shoot elongation, wilting, overall chlorosis, and death (Alexander *et al.* 1988).

PATHOGEN TRANSMISSION

Most conifer root diseases cause expanding centers of dead and dying trees, and therefore, show the clumped tree distribution typical of soil borne pathogens (Carlson 1994). Unlike other root diseases, trees with PRD are scattered within plantations, suggesting an association with insect vectors (Nevill and Alexander 1992b). Horner (1985) most frequently isolated the pathogen from the root collar region, with isolation success decreasing distally from that location. This suggests that *L. procerum* is introduced at the root collar, not through the roots or root grafts with adjacent trees. Early studies by Lewis *et al.* (1987) further indicated this pathogen is not soil borne. In addition, the close association of these types of fungi with subcortical arthropods (Harrington 1988), suggest *L. procerum* is insect vectored.

Stems of diseased trees were found to be infested with *H. pales, P. nemorensis,* and bark beetle species in the genera *Pityogenes, Orthotomicus, Xyleborus,* and *Hylastes* (Lackner and Alexander 1984). Lewis and Alexander (1986) investigated the role of insect vectors of *L. procerum* by surveying ten *P. strobus* Christmas tree plantations. Sixty-four percent of the weevil species present were contaminated with the fungus. They found < 1% of the bark beetle species to be carrying the fungus. Lewis and Alexander (1986) further demonstrated both weevil species could transmit *L. procerum* to pine bolts by placing the insects in a controlled, closed environment with the bolts. Nevill and Alexander (1992b) suggest that as the occurrence of PRD increases in a plantation, the percentage of weevils contaminated with *L. procerum* conidia also increases. Population studies in Virginia Christmas tree plantations found *H. pales* carried *L. procerum* five times more frequently than *P. nemorensis* (Nevill and Alexander 1992b).

Recent studies in Virginia have shown *L. procerum* is routinely carried by two weevil species, *H. pales* and *P. nemorensis* (Nevill and Alexander 1992b). Nevill and Alexander (1992a) further demonstrated the ability of naturally and artificially contaminated weevils to transmit the pathogen to *P. strobus* seedlings and saplings under controlled conditions. Adult weevils breed in dead and dying trees (Anderson 1980, Lynch 1984), and it appears likely that the brood and adults become contaminated in diseased trees or stumps. The sticky conidia of *L. procerum* are known to adhere to insects (Harrington 1988), and therefore may be transmitted to healthy trees via the feeding activities of contaminated weevils.

Hylobius pales is believed to be the principle vector of *L. procerum* to *P. strobus* for two reasons: 1) a much higher percentage of *H. pales* are found to carry the fungus and 2) the feeding activity of *H. pales* is more conducive to successful inoculation (Lynch 1984). *Hylobius pales* transmits the pathogenic fungus to Christmas trees through cambial feeding which is likely to occur at the root collar region of the infested host. Recovery patterns of the pathogen from colonized wood suggest inoculation in this region (Horner 1985). *Pissodes nemorensis* feeds primarily on the shoot tips and terminals; areas usually devoid of the pathogen (Horner 1985, Johnson and Lyon 1991).

OBJECTIVES

1) To identify effective trapping techniques for sampling *H. pales* and *P. nemorensis* populations in Virginia Christmas tree plantations.
2) To evaluate the effect of vegetation management treatments and trap rotation on the trap catch of *H. pales* and *P. nemorensis.*
3) To determine the seasonal activity of both weevil species.
4) To determine if trap catch can be positively correlated with seedling damage to provide a risk rating system.
5) To use mark-and-recapture techniques for assessing the efficacy of attractive baits and traps for capturing *H. pales* adults.
6) To measure the positive response of walking weevils in three age classes to a defined source of simulated host odors in a laboratory bioassay.

Each chapter is written independently in scientific journal format, resulting in some repetition when describing life histories, control, and experimental procedures used in these studies.

CHAPTER 2

Evaluation of Factors Influencing Trap Catch of *Hylobius pales* and *Pissodes nemorensis* in Virginia Christmas Tree Plantations

INTRODUCTION

The pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae) is a major regeneration pest of pine plantations in the southern United States (Fox and Hill 1973, Doggett *et al.* 1977). It also has been implicated as the principle vector of the pathogenic fungus *Leptographium procerum* (Kendr.) Wing. to pine trees, resulting in the development of procerum root disease (PRD) in some eastern white pine (*Pinus strobus* L.) plantations (Nevill and Alexander 1992a, b). The deodar weevil, *Pissodes nemorensis* Germar (Coleoptera: Curculionidae) is a similar pest of cedar and *Pinus* spp. in the eastern United States and southeastern Canada (Johnson and Lyon 1991). The role of *P. nemorensis* in the transmission of PRD is believed to be secondary to that of *H. pales* (Lackner and Alexander 1983, 1984, Lewis and Alexander 1986, Lewis *et al.* 1987).

Adult *H. pales* cause seedling mortality in Christmas tree plantations just as they do in newly established forest plantations. Mortality has been recorded as high as 90%, with losses commonly exceeding 40% following the initial year of planting (Peirson 1921, Nord *et al.* 1982). Seedlings can sustain significant levels of feeding; however, their form and symmetry is affected, resulting in culled trees at harvest (Holt and Bramble 1956). Economic losses are higher in selectively cut plantations (choose and cut farms) which provide new breeding material annually (Corneil 1981).

An effective sampling strategy could help determine when serious infestations are imminent. If a correlation of weevil density with seedling damage is established, it may be possible to develop a risk rating system (Waters *et al.* 1985). Calendar spray applications, preplant seedling dips, and stump applications of Lindane® are commonly recommended as preventative measures (Day *et al.* 1995). However, Lindane® is highly toxic, persistent, and carcinogenic to laboratory animals (Rieske and Raffa 1993). A risk rating system could reduce our reliance on insecticide use by limiting the application of these materials to damaging infestation levels.

Several techniques have been used to sample weevil populations (Lynch 1984). Studies show traps incorporating natural pine material are most effective in trapping weevils. However, the use of this material has inherent disadvantages, being more costly and labor intensive to provide than dispensers containing attractive synthetic volatiles. In order to overcome the associated drawbacks, Tilles *et al.* (1986a, b) designed a PVC pitfall trap baited with host volatiles for sampling European pine weevil, *Hylobius abietis* (L.), populations in Sweden. This trapping regime has been used successfully in the Lake States and Europe (Tilles *et al.* 1986a, b, Nordlander 1987, Raffa and Hunt 1988, Hunt and Raffa 1989, Rieske 1990, Rieske and Raffa 1991, 1993), but has shown limited success in Great Britain (Wilson and Day 1995) and Virginia (J. Gray, personal communication).

There have been several attempts to correlate the trap catch of *Hylobius* spp. with seedling damage in order to provide a risk rating system. Wilson and Day (1994) found trapped weevil numbers weakly correlated with observed damage, possibly because only a fraction of the damage-causing weevils are susceptible to traps. Frazier (1969) and Lawrence (1975) found no relationship between the number of weevils trapped and subsequent seedling mortality. Doggett *et al.* (1977) found a correlation between the number of weevils trapped and seedling mortality but recommended further studies to strengthen the correlation. Rieske and Raffa (1993) found that the number of female *H. pales* was weakly correlated with damage estimates of harvestable trees the following year, based on foliar discoloration severity rankings.

The height and density of secondary ground cover is known to affect weevil access to target seedlings (Frazier 1969, Stadnitskii 1978, Wilson and Day 1994); however, results are often contradictory. The effect of ground cover on trap catch has not been determined, but may be similar to that observed with seedlings.

In this study, field assays were performed during 1994 and 1995 to identify an effective trapping technique for sampling *H. pales* and *P. nemorensis* populations in Virginia Christmas tree plantations. The effects of vegetation management and rotation policy on trap catch were evaluated. The seasonal distributions of both species were determined. Investigations were performed to determine if the number of trapped weevils could be positively correlated with seedling damage in Virginia Christmas tree plantations. Three different vegetation management treatments were evaluated for their effect on feeding activity.

MATERIALS AND METHODS

Experiment 1: *Development and evaluation of trapping strategies 1994*

Four *P. strobus* Christmas tree plantations located in Floyd and Montgomery Counties were selected as study areas. All were of harvestable age (5-8 yrs) with no recent history of insecticide or pesticide application. Two plantations were active Christmas tree farms, while the remaining two were managed primarily for nursery stock. In one of the nursery stock plantations, PRD was epidemic and provided abundant breeding sites for *H. pales*. Three plots were established in this plantation, and one in each of the others, for a total of 6 plots. Each plot (approx. 1.9 ha) was further subdivided into subplots with the following type of vegetation management: 1) natural ground cover (unmanaged); 2) natural ground cover mowed twice during the growing season; and 3) natural ground cover treated with two applications of Roundup® (glyphosate) herbicide: one in late spring and another in mid-summer. Each subplot was further divided into four sub - subplots by trap type and rotation treatment (Figure 1). Rotated traps were randomly relocated within each sub-subplot following biweekly (2 week interval) collection. It is hypothesized that since *Hylobius* spp. tend to aggregated around stump sites, stationary traps may trap out isolated weevil populations around those sites, or report inaccurate trap catch estimates over time.

Two traps were placed in each sub-subplot according to trap type for that specific location. Trap placement was randomly assigned as was subplot location. Traps were placed at least 25 m apart, between trees, to reduce trap interference and disturbances associated with the active management of Christmas tree plantations.

Two trap types were examined in this experiment. A PVC pitfall trap, similar to that used by Tilles *et al.* (1986a), was constructed from 21.5 cm lengths of 10 cm. dia., 6.35 mm thick, white PVC plastic drainpipes. Eight 9 mm entrance holes were drilled around the circumference of the trap, 5 cm from the top. Each trap was placed vertically in the ground deep enough for entrance holes to be flush with the soil surface. A 25 ml glass vial containing approximately 23 ml of a 5:1 ethanol-and-turpentine mixture (release rate: 628.6 mg/24 h at 24.5° C) was hung on a thin aluminum wire within each trap (Rieske and Raffa 1991). A soapy water mixture was poured into the bottom 3 cm of the trap to prevent weevil escape. Both ends of the trap were capped and the location of each trap was clearly delineated by a fluorescent flag.

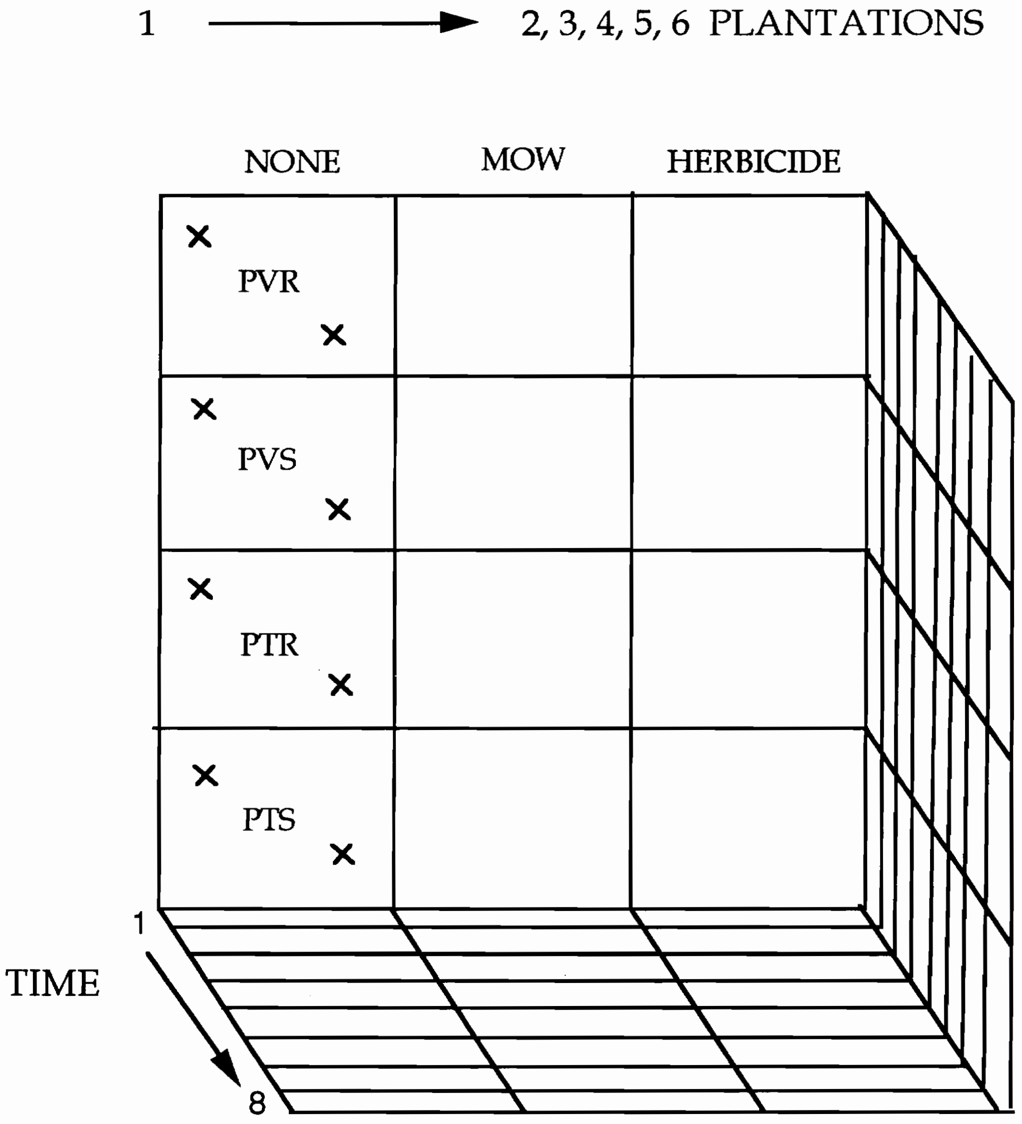

Figure 1. Experimental design of weevil trapping studies conducted in Floyd and Montgomery Counties, Virginia, 1994. Four trap types were evaluated: 1) PVR = PVC pitfall rotated; 2) PVS = PVC pitfall stationary; 3) PTR = pit trap rotated; and 4) PTS = pit trap stationary. Trap locations are delineated by X.

A pit trap, similar to that used by Doggett *et al.* (1977), was also tested. A freshly-cut *P. strobus* bolt, 8 to 12 cm in diameter and 30 cm long was placed in a shallow pit in close proximity to the base of a healthy *P. strobus* to attract foraging weevils. The bolt was treated with Lindane® in order to prevent weevil escape. A 25 ml glass vial containing approximately 23 ml of the 5:1 ethanol-and-turpentine mixture was added as additional attractant material. The trap was covered with a black tile and fresh *P. strobus* slash to prevent desiccation.

All traps were checked every two weeks. Specimens were placed in labeled vials containing 70% ethanol for identification and tally in the laboratory. Synthetic volatiles, pine billets, and slash were removed from the traps and replaced with fresh material during collection.

The experimental design was a split-split plot (RCBD, RCBD) in time (Steel and Torrie 1980, Hicks 1982). A test of normality was performed on the trap catch data using PROC UNIVARIATE (SAS Institute 1989), showing the data deviated significantly from a normal distribution. The data were square root transformed and analyzed using PROC GLM and the Tukey test for mean separations (SAS Institute 1989).

Experiment 2: *Field assay of two trap types 1995*

Study areas were selected in the same plantations sampled in 1994. Each plantation was divided into six 0.16 ha plots. Four stationary traps (2 pit, 2 PVC pitfall) were randomly located in each plot (Figure 2). Traps were placed at least 25 m apart, between trees, to reduce trap interference and disturbances associated with the active management of Christmas tree plantations.

Two trap types were examined in this experiment. A PVC pitfall similar to that tested during 1994 was examined. An approximate 20:1 vaseline and baby oil mixture (Johnson and Johnson, Skillman, NJ) was used to prevent weevil escape instead of the soapy water mixture used in 1994. Although Fluon® (E. I. duPont de Nemours, Wilmington, DE) is commonly recommended to coat trap interiors (Rieske and Raffa 1990, 1991), we have found it difficult to obtain commercially and unreliable in preventing weevil escape. The pit trap tested was identical to that examined during 1994.

All traps were checked weekly for the presence of weevil species, and insects were removed from the plantations. All adult *H. pales* were sexed. Synthetic volatiles, pine billets, and slash were removed from the traps and replaced with fresh material every two weeks.

The experimental design was a split plot replicated over time (Steel and Torrie 1980). The data were analyzed using PROC GLM and the Tukey test for mean separations (SAS Institute 1989).

Experiment 3: *Seedling survey*

In 1994, twenty improved, disease-free, *P. strobus* (2+0) seedlings were planted in each subplot (5 per sub-subplot) during late March, for a total of 360

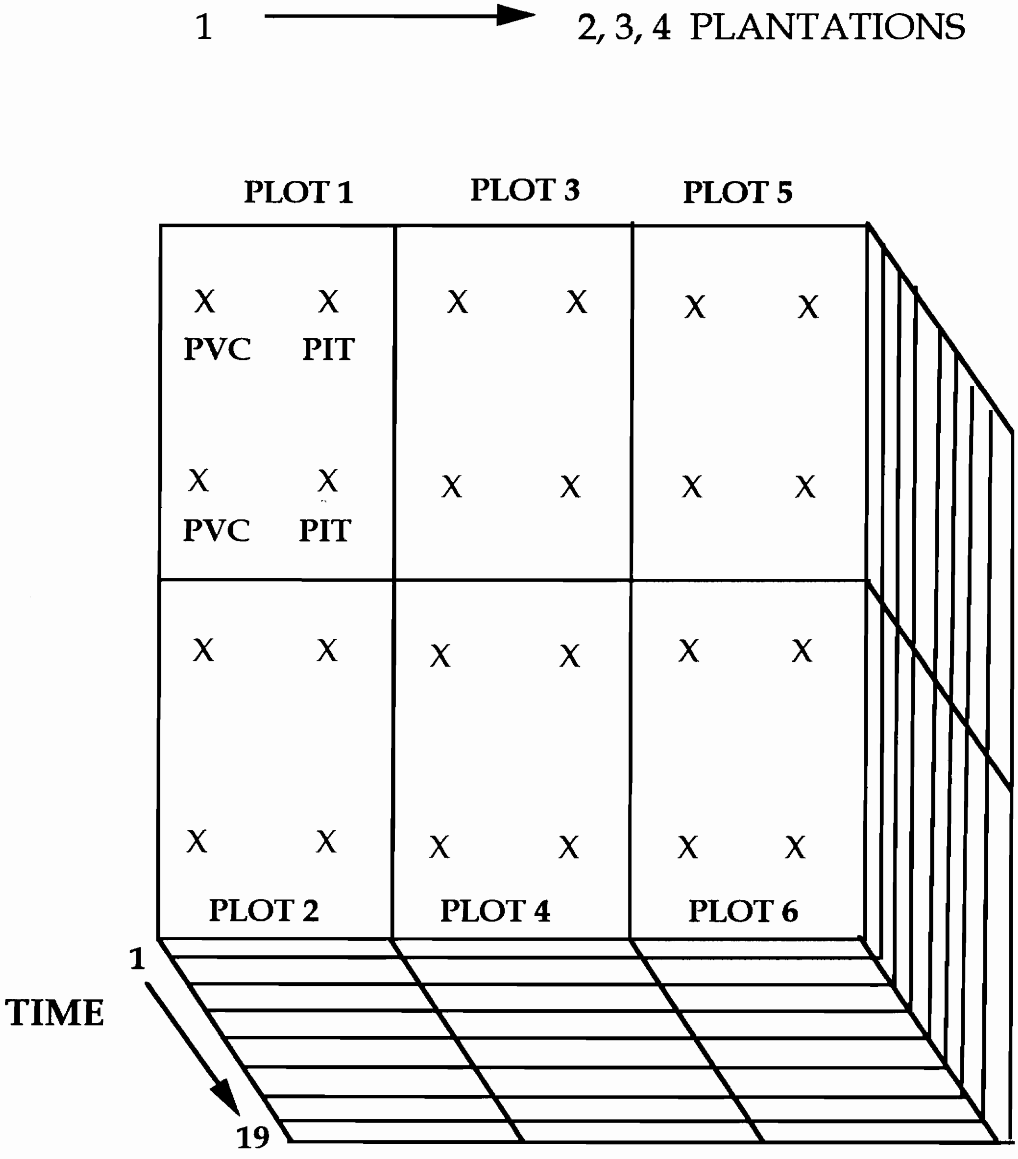

Figure 2. Experimental design of weevil trapping studies conducted in Floyd County, VA, 1995. Two trap types were evaluated: 1) PVC pitfall traps; and 2) pit traps. Trap locations are denoted by X.

seedlings. Seedlings were examined biweekly for weevil feeding, weevil-induced mortality, and natural mortality from May 30 - October 1, 1994. The amount of feeding was recorded from the root collar region to the apical meristem, by measurement of wound areas with a transparent (mm^2) dot grid. In 1995, fifteen improved, disease-free, *P. strobus* seedlings (2+0) seedlings were planted in each plot in late March, for a total of 360 seedlings in this experiment. Seedlings were monitored weekly from May 10 - September 14, 1995 using the same techniques in 1994.

The 1994 data were analyzed using PROC GLM , and the Tukey test for mean separations to determine the effect of vegetation management on feeding activity (SAS Institute 1989). Correlation attempts were first performed by hand drawn graphs correlating trap catch with seedling mortality. Since few seedlings suffered weevil-induced mortality, further attempts were made to correlate trap catch with weevil feeding by proportion and amount. If a sufficient correlation was found, the relationship was analyzed using the Pearson's correlation coefficient (SAS Institute 1989).

RESULTS

Experiment 1: *Development and evaluation of trapping strategies 1994*

There was a significant treatment effect for main effects ($F_{12, 574} = 1.25$; $P < 0.004$). Labor intensive pit traps, using natural host material and synthetic volatiles, caught significantly more *H. pales* than PVC pitfall traps baited with synthetic volatiles alone (Table 3). Trap rotation did not affect the number of weevils captured (Table 3). Vegetation management had no effect on trap catch ($F_{2, 574} = 1.64$; $P = 0.24$).

Total trap catch of *H. pales* was 385. Pit traps caught 3.9 times (n=306) more *H. pales* than PVC pitfall traps (n=79). Time was significant ($F_{7, 574} = 7.19$; $P < 0.0001$) and resulted in a bimodal distribution with an early peak occurring in mid-June and another in early September (Figure 3).

A significant trap x time interaction ($F_{21, 418} = 5.79$; $P < 0.0001$) was observed for *P. nemorensis,* therefore the data were further analyzed for time. Vegetation management had no effect on trap catch ($F_{2, 418} = 0.06$; $P = 0.94$). No significant differences in trap catch were observed between stationary and rotated traps ($F_{1, 208} =1.22$; $P = 0.27$). Total trap catch of *P. nemorensis* was 250, with pit traps accounting for 98% (n=245) of this figure. Pit traps caught significantly more *P. nemorensis* than PVC pitfall traps during 7 of the 8 sampling periods (Figure 4). The seasonal trap catch of *P. nemorensis* resulted in a bimodal distribution similar to that of *H. pales,* with both major peaks occurring two weeks earlier (Figure 4).

Experiment 2: *Field assay of two trap types 1995*

Both sexes of *H. pales* responded similarly in their attraction to the trapping regimes ($F_{1, 3569} = 9.45$, $P = 0.27$). There was a significant trap x time

Table 3. Mean trap catch (± S.E.) of *H. pales* by four different trap types evaluated from May 30 to October 1, 1994 in Floyd and Montgomery Counties, VA.

Trap Type [a]	N	$\bar{x}$ ± S.E. [b]
PTR	288	0.54 ± 0.05 a
PTS	288	0.53 ± 0.06 a
PVR	288	0.16 ± 0.06 b
PVS	288	0.11 ± 0.03 b

[a] PTR, pit trap rotated; PTS, pit trap stationary; PVR, PVC trap rotated; PVS, PVC trap stationary

[b] Means followed by the same letter are not significantly different (P > 0.05; Tukey test).

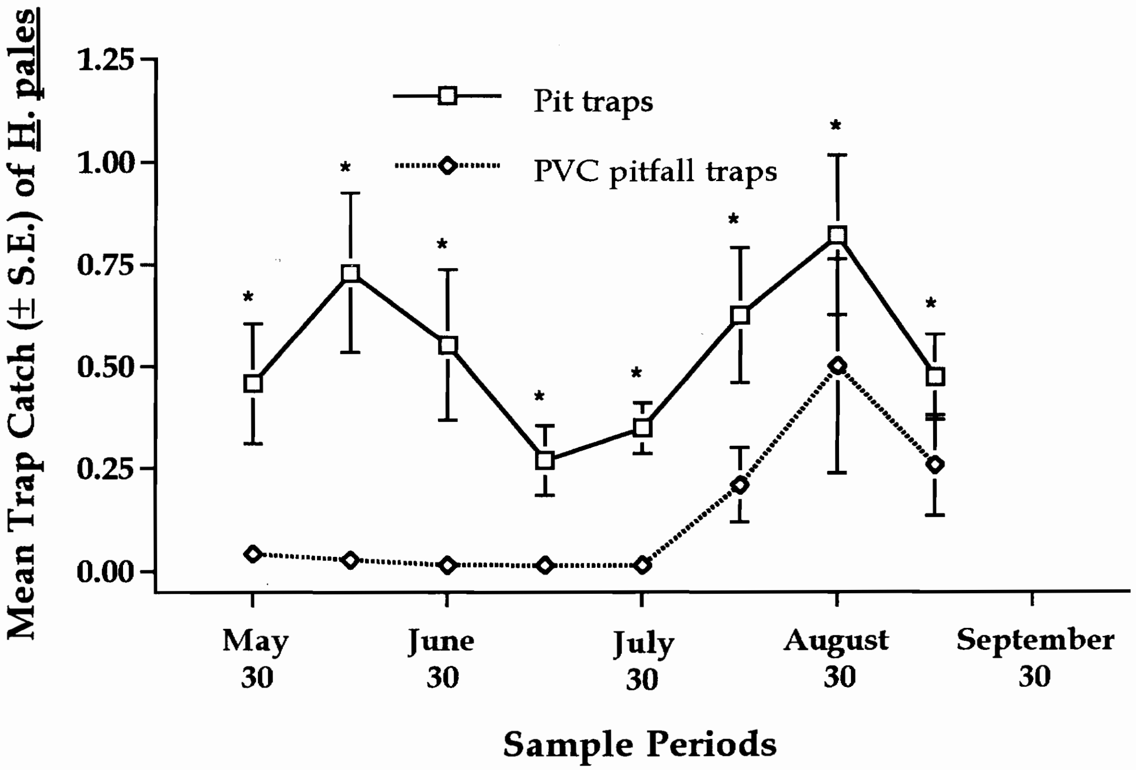

Figure 3. Mean trap catch (± S.E.) of *H. pales* in Virginia Christmas tree plantations, 1994. Asterisks (*) denote a significant difference between treatment means for each sample date ($P < 0.05$; Tukey test).

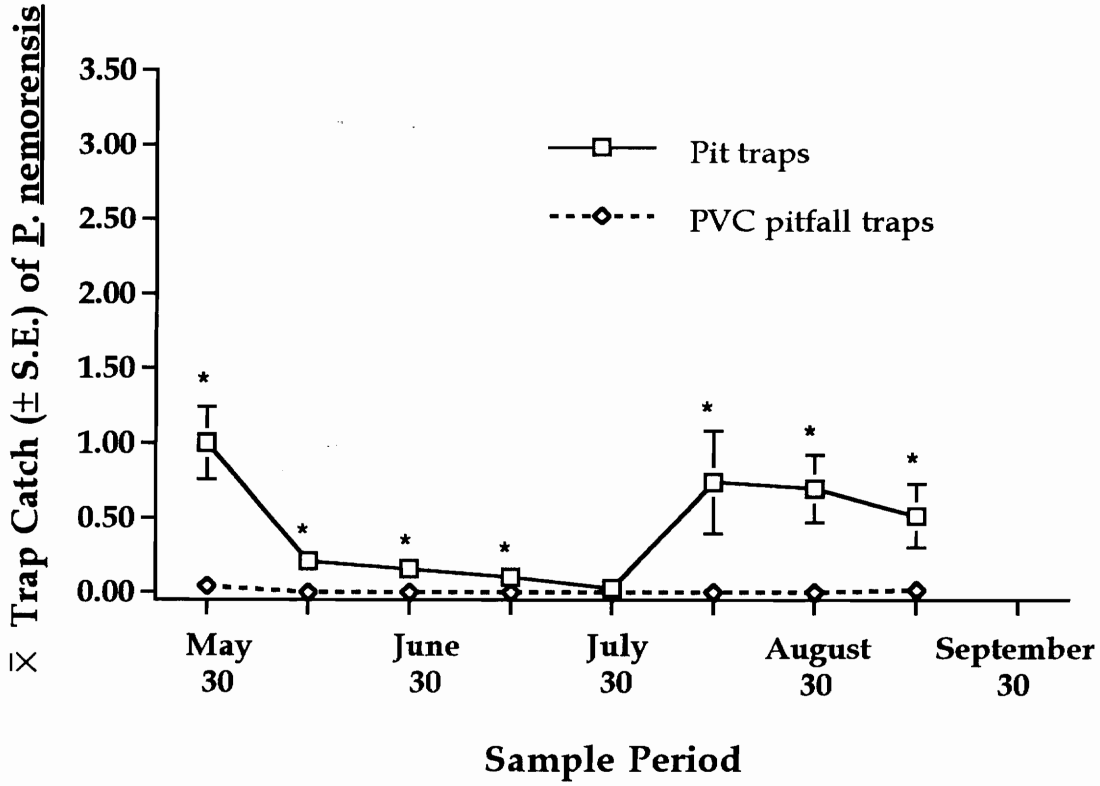

Figure 4. Mean trap catch (± S.E.) of *P. nemorensis* in Virginia Christmas tree plantations, 1994. Asterisks (*) denote a significant difference between treatment means for each sample date ($P < 0.05$; Tukey test).

interaction ($F_{18, 3569} = 9.45$, $P < 0.0001$), therefore the data were further analyzed for time.

Total trap catch of *H. pales* was 617. Pit traps caught 5.3 times (n=519) more *H. pales* than PVC pitfall traps (n=98). Pit traps caught significantly more *H. pales* during 11 of the 19 sampling periods (Figure 5). In the remaining periods, there were no significant differences between the two trapping regimes. The majority of these periods occurred at the end of the study; a time when trap catches were extremely low for both traps. *Hylobius pales* adults were most numerous during May, June, and July, with general reductions in trap catch observed throughout the remaining sampling periods (Figure 5).

A significant trap x time interaction ($F_{18, 1783} = 17.31$, $P < 0.0001$) was observed for *P. nemorensis*, therefore the data were further analyzed for time. Total trap catch was 623, with pit traps accounting for 97% of this figure. Pit traps caught significantly more weevils during 17 of the 19 sampling periods (Figure 6). The seasonal trap catch of *P. nemorensis* resulted in a distribution similar to that observed for *H. pales* in 1995 (Figure 6).

Experiment 3: *Seedling survey*

In 1994, 14.4 % (n=52) of the seedlings suffered some degree of weevil feeding (Figure 7). Thirteen percent (n=7) of those seedlings died as a result of the injury. Many seedlings sustained repeated feeding by *H. pales* throughout

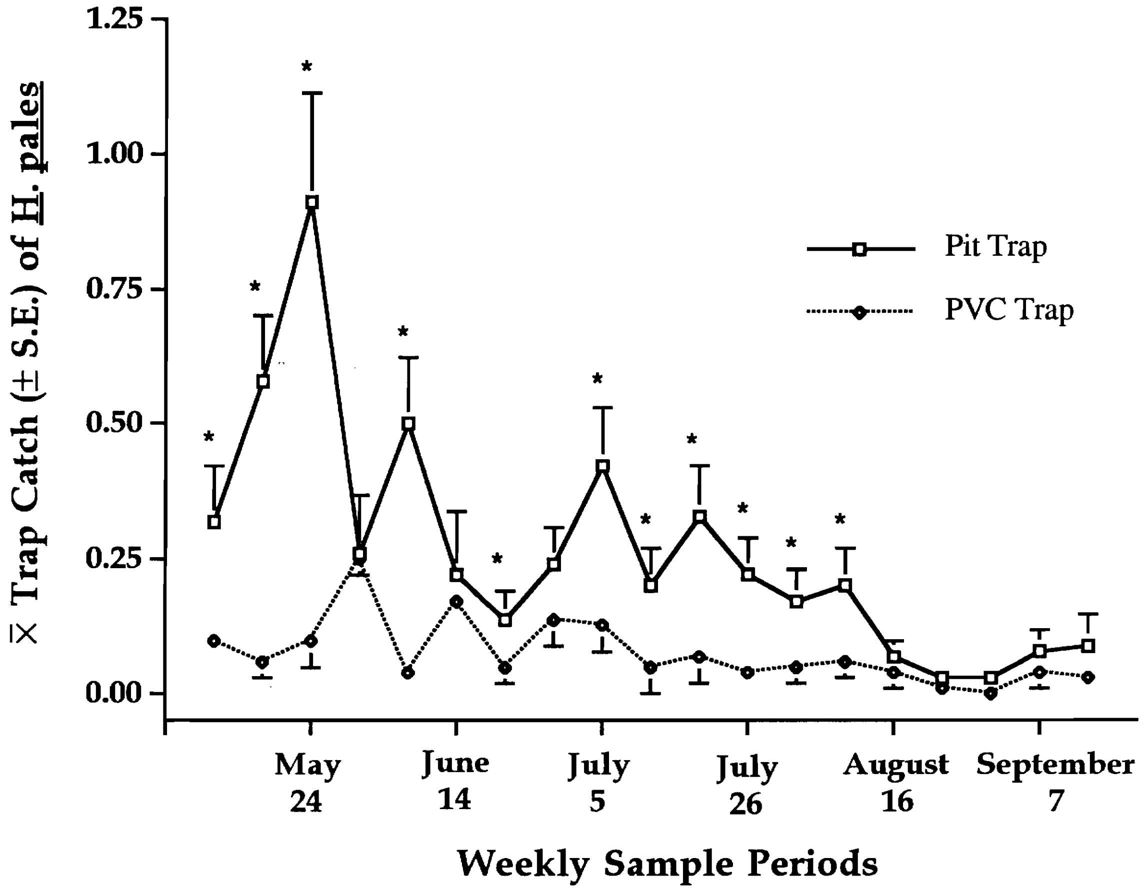

Figure 5. Mean trap catch (± S.E.) of *H. pales* in Virginia Christmas tree plantations, 1995. Asterisks (*) denote a significant difference between treatment means for each sample date ($P < 0.05$; Tukey test).

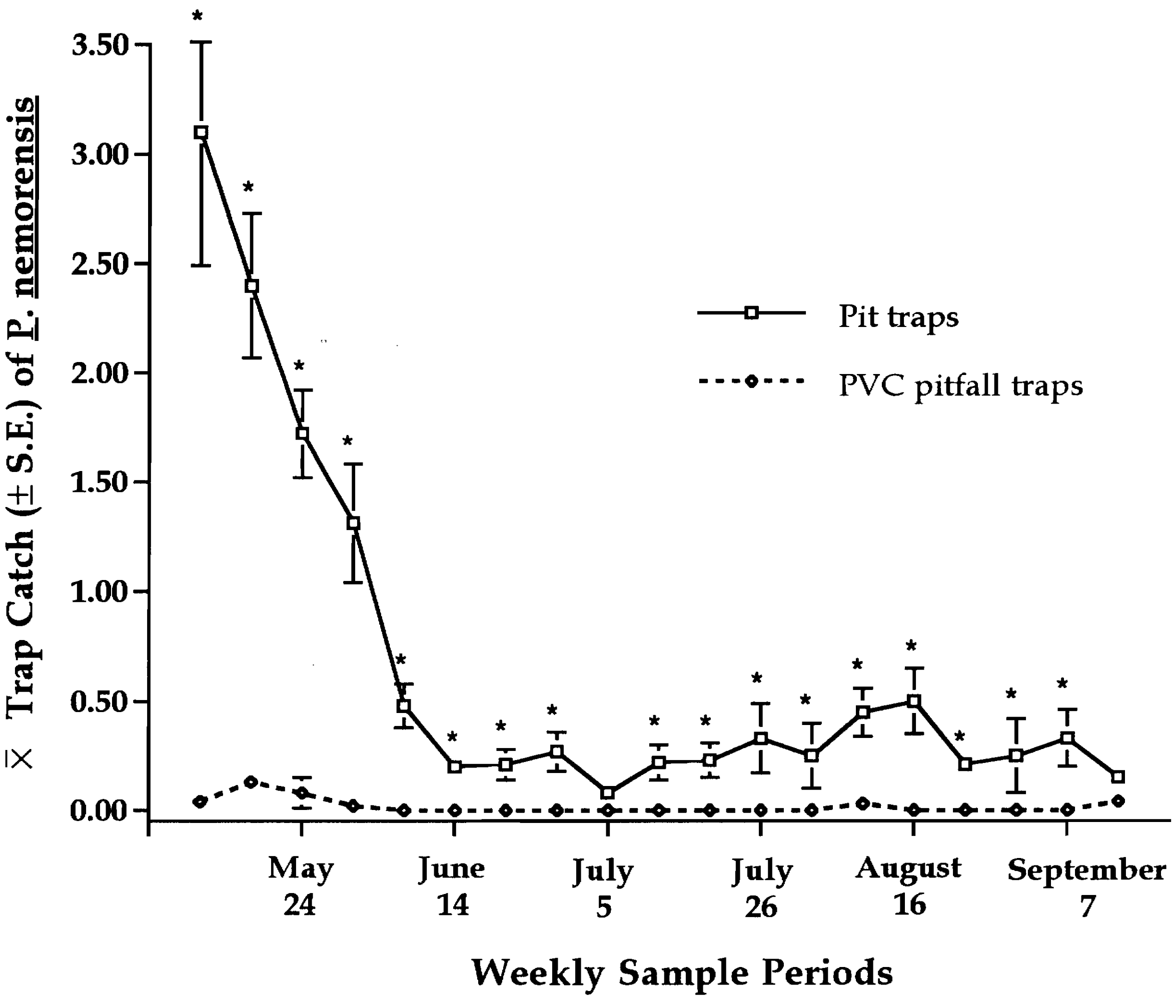

Figure 6. Mean trap catch (± S.E.) of *P. nemorensis* in Virginia Christmas tree plantations, 1995. Asterisks (*) denote a significant difference between treatment means for each sample date ($P < 0.05$; Tukey test).

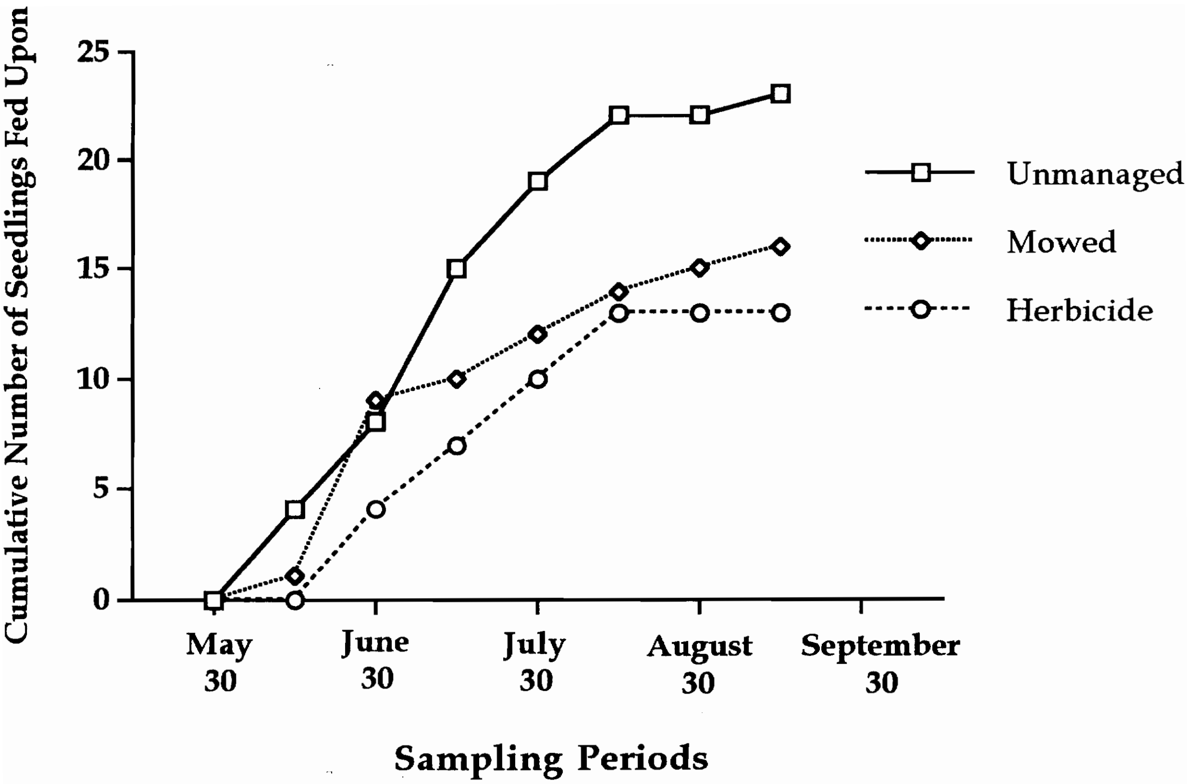

Figure 7. Cumulative number of seedlings with *H. pales* feeding injury in Virginia Christmas tree plantations, 1994.

the growing season. Fifteen percent (n=55) were afflicted with mortality not associated with weevil feeding. Time was significant ($F_{7, 115} = 3.18$, $P < 0.004$). *H. pales* adults fed at higher rates during sample period 3 (30 June) than during sample period 1 (30 May) (Figure 8). All remaining sample periods had no significant differences among their means. A significant vegetation management treatment was observed ($F_{2, 115} = 3.21$, $P < 0.04$) (Table 4). The proportion of seedlings fed upon was significantly greater in vegetation-unmanaged plots than in herbicide treated plots (Table 4).

In 1995, 4.7% (n=17) of the seedlings suffered some degree of weevil feeding, but only one died as a result of the associated injury. Mortality non-associated with weevil feeding claimed 25% of the test population.

No correlation was found between trap catch and seedling damage within or among sample periods, or between yearly trap catches and seedling damage. Correlation attempts were unsuccessful for all trap types and combinations there of in three separate vegetation management treatments.

DISCUSSION

Hylobius pales males and females responded similarly in their attraction to traps. These equivalent responses suggest that the volatiles emanating from baits must function as both feeding and ovipositional cues. Similar results have been found in field studies conducted in Wisconsin Christmas tree plantations

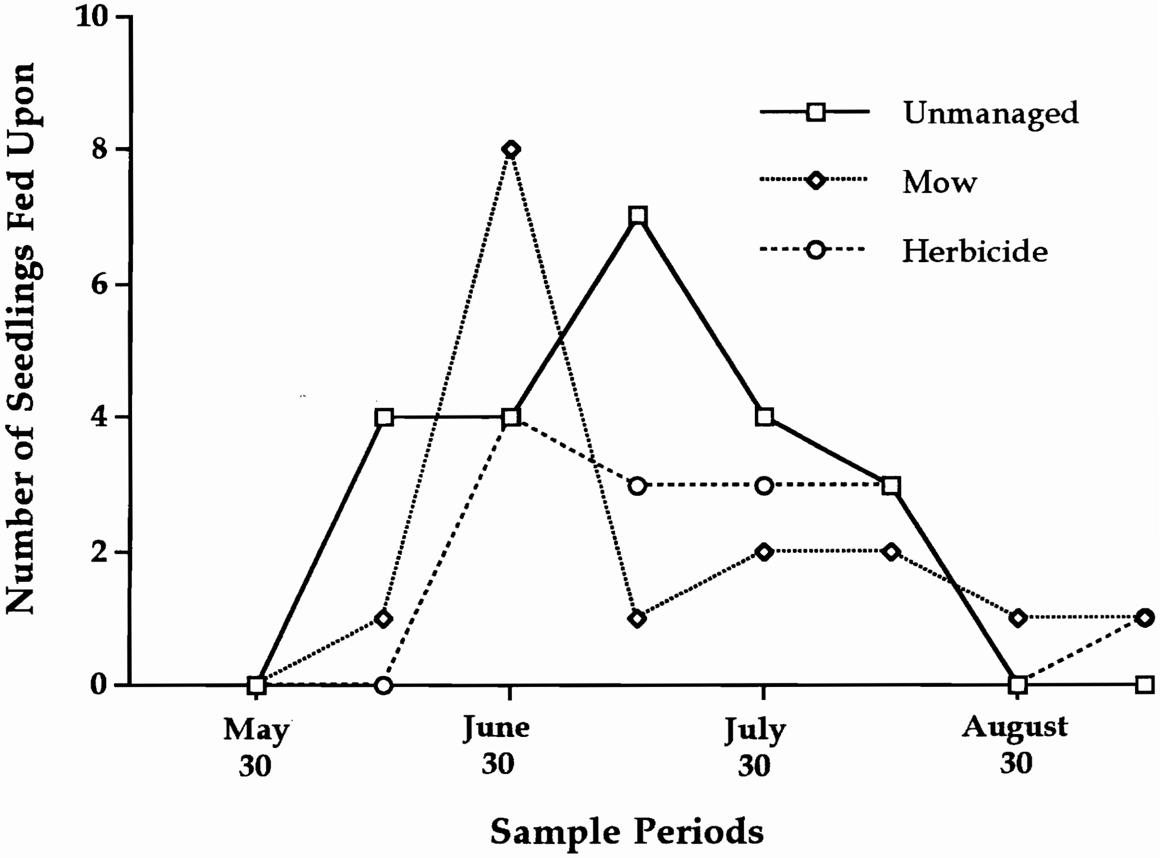

Figure 8. Number of seedlings afflicted with *H. pales* feeding among three different vegetation management treatments in Virginia Christmas tree plantations, 1994.

Table 4. Mean number (± S.E.) of *P. strobus* seedlings fed upon by *H. pales* adults in Virginia Christmas tree plantations, 1994.

Vegetation Mgmt.	**N**	$\bar{x}$ **± S.E.** [a]
Unmanaged	48	3.65 ± 0.86 a
Mow	48	2.39 ± 0.61 ab
Herbicide	48	1.45 ± 0.45 b

[a] Means followed by the same letter are not significantly different ($P > 0.05$; Tukey test).

(Rieske and Raffa 1991).

In this study, trap rotation had no effect on trap catch. This suggests that these trapping regimes do not trap out isolated populations within a plantation, or provide inaccurate trap catch estimates. We can assume equal attraction to traps for all weevils within a plantation, and therefore suggest it is unnecessary to rotate traps in order to successfully quantify weevil populations.

Vegetation management had no effect on trap catch. Although tree farmers commonly manage weed species with herbicide applications or by mowing, these activities do not appear to influence weevil attraction to baited traps. However, vegetation height and density may affect weevil access to target seedlings. The risk of damage to seedlings by weevil feeding depends on several factors. Most important are factors influencing population dynamics of the insect, for example, the availability of suitable forage sites to support breeding weevils and their developing brood. Stadnitskii (1978) found lower vegetation heights corresponded with increased seedling damage by *H. abietis* in *P. sylvestris* plantations. Since *Hylobius* spp. rely primarily on tactile locomotion it seems that a heavy ground cover could provide a physical barrier or distort the reception of olfactory cues provided by the seedling. However, our results show that the amount of secondary ground cover corresponds directly with observed damage (Table 4). Frazier (1969) surveyed ten Virginia

P. taeda regeneration sites, and found seedlings planted on litter covered ground were fed upon at significantly higher rates than those on bare mineral soil. Salom *et al.* (1986) found the survivorship of *H. pales* immatures decreased rapidly with temperatures above 25° C, and development ceased above 32° C. Summer temperatures on exposed soil surfaces in Virginia are often in excess of 35° C. The increase in feeding activity with respect to increased ground vegetation height and density may be due to the creation of a more favorable microenvironment (cooler and more temperate) resulting from reductions in solar radiation by competing vegetation.

Natural host-baited pit traps were more effective in capturing *H. pales* adults than synthetically-baited PVC pitfall traps. During the two year study there were few sample periods when pit traps did not catch significantly more weevils than PVC pitfall traps. The ineffectiveness of PVC pitfall traps does not result from weevil escape, as preliminary laboratory studies indicated that weevils are retained in the traps. In addition, the entrance holes are of sufficient diameter to allow unabated weevil access. The different components of each trapping system will be reviewed in detail in chapters 3 and 4. The use of the PVC pitfall trapping system would be highly desirable; however, our results suggests we must rely on the use of pit traps for monitoring weevil populations in Virginia until an appropriate system for implementing the PVC pitfall trapping regime can be developed.

The life cycle of *H. pales* in the southern United States is characterized by overlapping generations and split adult emergence periods (Lynch 1984). In 1994, *H. pales* adults were found most active in June and early September (Figure 3). Since, *H. pales* overwinters as larvae in fresh stumps and adults in the leaf litter, there tends to be a long period in late spring and early summer when a large portion of adults are captured. Such trends are commonly reported in the literature (Carter 1916, Anderson 1980, Lynch 1984). However, the second peak is not usually reported, and probably results from a late brood emergence produced by early emergent adults (Speers 1974). The shearing of *P. strobus* Christmas trees coincides with the decrease in trap catch observed between the two peaks. Shearing activities produce additional forage material, and a large increase in the release of resinous volatiles that may be more attractive to weevils than those produced by the traps. However, in these studies shearing practices were not uniform among sites, and therefore no statistical analysis could be performed on the effect on trap catch. In 1995, *H. pales* adults were most numerous in May, June, and July (Figure 5). These results directly support those observed by Anderson (1980) in the same geographic area. The lack of a second peak in early fall 1995 may have resulted from unfavorably hot and dry weather conditions present in the area during the last five sampling periods. These conditions may have had a detrimental effect on weevil survival and movement. In addition, severe conditions often increase the proportion of stressed trees within a plantation (Rieske and Raffa 1993). The release of ethanol by stressed trees may have been very attractive to *H. pales* adults, thus reducing the effectiveness of traps.

In 1994, *P. nemorensis* adults were most active in late May and mid to late August (Figure 4). Anderson (1980) reports finding *P. nemorensis* adults most active in March, April, and May in southwest Virginia Christmas tree plantations, and caught few adults after late June. In 1995, *P. nemorensis* was most numerous from May through mid June (Figure 6). The female dies shortly after laying all her eggs (Finnegan 1958), and therefore adults are most commonly found prior to the peak ovipositional period. However, a small percentage of females are said to have an extended ovipositional period that carries into the fall months (Hard 1962). A lower percentage of females with extended ovipositional periods, in conjunction with unfavorable weather conditions as mentioned previously, may explain the lack of *P. nemorensis* adults present in the early fall of 1995.

Anderson (1980) found 50% of seedlings surveyed suffered some degree of weevil feeding in Virginia Christmas tree plantations. Fifty-two percent of those seedlings died as a result of the associated injury. Seedlings were attacked immediately following planting and the incidence of feeding injury declined sharply after mid-June. Our results show few significant differences in feeding injury during the 1994 study, suggesting no apparent trends between seasons and feeding activity exist. We found a lower percentage of seedlings suffering feeding or weevil-induced mortality relative to that observed by Anderson (1980). However, Anderson's (1980) studies had a much smaller sample size (n=96), and the total number of seedlings fed upon was quite similar between the two studies.

In forest management, the rate of plant mortality rather than the number of seedlings sustaining sub-lethal damage is of primary concern. However, in Christmas tree plantations any feeding damage can affect the form and symmetry, resulting in culled trees at harvest. Attempts to correlate seedling damage with trap catch were unsuccessful, supporting the observations of several other researchers (Frazier 1969, Lawrence 1975, Wilson and Day 1994). Sampling was initiated prior to seedling damage being observed and continued after it started to subside. It seems improbable that weevils could have migrated to the site and gone undetected. Perhaps the relative attractiveness of seedlings and stump sites to weevils was greater than that provided by the traps, thereby allowing a significant proportion of the population to go undetected.

CHAPTER 3

Mark and Recapture Studies of *Hylobius pales* (Herbst) (Coleoptera: Curculionidae) For Measuring Trap Efficacy

INTRODUCTION

The pales weevil, *Hylobius pales* (Herbst) (Coleoptera: Curculionidae), first considered a pest of pine in the northeastern United States and southeastern Canada (Carter 1916, Peirson 1921), has now become a major regeneration pest of pine plantations in the southern United States (Fox and Hill 1973, Doggett *et al.* 1977). Larvae feed and develop on the cambium, phloem and xylem tissues of stumps and roots. Adults generally feed at night or on cloudy days and oviposit in the roots and root collars of stressed or recently killed pine trees, as well as other coniferous species (Carter 1916, Peirson 1921, Lynch 1984). They are attracted to the resinous volatiles produced by dead and dying trees. Possible indirect damage may result from *H. pales* transmitting the pathogenic fungus *Leptographium procerum* (Kendr.) Wing. to pine trees, leading to the development of procerum root disease (PRD) in some eastern white pine (*Pinus strobus* L.) plantations (Nevill and Alexander 1992a, b). With additional increases in the planting of monoculture pines for timber and pulp, and the outbreak of PRD in some Christmas tree plantations, the management of *H. pales* has become increasingly important.

Conventional control measures incorporate the use of insecticides

(Lynch 1984, Rieske and Raffa 1991). Cultural control emphasizes timely harvests, site preparations, and delayed planting strategies that reduce attractiveness of the site to feeding weevils (Nord *et al.* 1982). Effective sampling could help determine when serious infestations are imminent, and reduce our reliance on insecticide usage by limiting applications to damaging infestation levels.

Several techniques have been used to sample *H. pales* populations. Studies show traps incorporating natural pine material are effective in trapping weevils. Doggett *et al.* (1977) found pit traps consisting of freshly-cut pine bolts, 7.5 to 12.5 cm in diameter and 61 cm long to be effective in sampling *H. pales* populations in North Carolina.

Natural host material used in various trapping methods has inherent disadvantages, and is more costly and labor intensive than the use of dispensers containing synthetic host volatiles. In order to overcome these drawbacks, Tilles *et al.* (1986a, b) used a PVC pitfall trap baited with host volatiles (α-pinene and ethanol) for sampling European pine weevil, *Hylobius abietis* L., populations in Sweden. The trap was constructed from 20 cm lengths of 11 cm. dia., 2 mm thick, grey PVC plastic drainpipes. Rieske and Raffa (1991, 1993) used similar traps for trapping *H. pales*, the pitch-eating weevil, *Pachylobius picivorus* Germar, and the root collar weevil, *Hylobius radicis* Buchanan, in Wisconsin Christmas tree plantations and tested the response of these species to different volumetric ratios of ethanol and turpentine.

Previous studies have shown a 1:1 volumetric ratio of ethanol and turpentine will attract weevils, whereas blank traps or traps with either compound alone showed no attraction (Raffa and Hunt 1988, Hunt and Raffa 1989). Both ethanol and turpentine are reported as host cues for *Hylobius* spp. (Tilles *et al.* 1986a, b, Raffa and Hunt 1988, Hunt and Raffa 1989). Their synergistic effect make baits more attractive (Raffa and Hunt 1988, Hunt and Raffa 1989). Turpentine is comprised primarily of host monoterpenes of which α-pinene and β-pinene are the major constituents (Mirov 1961). However, it has been suggested that α-pinene and β-pinene are more suitable baits, as turpentine compositions vary from year to year and may actually contain constituents that act as deterrents when present (G. Nordlander, Swedish University of Agricultural Sciences, personal communication). Ethanol is a product of anaerobic plant and microbial metabolism and levels are known to vary considerably between species and seasons (Crawford and Baines 1977). Monoterpenes are believed to provide insects information on host species identification, while ethanol may signify the plant is stressed (Kimmerer and Kozlowski 1982). Rieske and Raffa (1991) found *H. pales* most attracted to ethanol-turpentine ratios ≥ 5:1 in field studies conducted in Wisconsin Christmas tree plantations. Both males and females responded similarly in their attraction to these compounds.

Mark-and-recapture studies have been used to assess insect dispersion and migration, evaluate trap efficacy, and estimate population dynamics for many insect species (Southwood 1978). This technique has been used to describe qualitatively *Hylobius* spp. movements within plantations (Corneil and Wilson 1984), and to describe quantitatively dispersion patterns, and estimate field populations (Rieske and Raffa 1990). Mark-and-recapture studies have not been used in any detail to assay the efficacy of various attractants and trap designs in capturing *H. pales* adults. Several underlying assumptions must be met when using this technique to establish population estimates (Caughley 1977, Southwood 1978); however, only the first assumption that the mark bear no adverse effect to the insect applies in these studies, because we are not concerned with the relative abundance of marked and unmarked individuals.

The objective of this study is to use mark-and-recapture techniques to assess the efficacy of various attractants and trap designs for capturing *H. pales* adults. The efficiency of both synthetically-baited PVC pitfall and natural host-baited pit traps were evaluated. In addition, several synthetic baits at different release rates were evaluated.

MATERIALS AND METHODS

Traps

Two trap types were tested: PVC pitfall and traditional pit traps. The PVC pitfall traps consisted of a modified version of those used by Tilles *et al.* (1986a, b) and were constructed from 21.5 cm lengths of 10 cm dia., 6.35 mm thick, white PVC plastic drainpipes. Eight 9 mm entrance holes were drilled around the circumference of the trap, 5 cm from the top. Both ends of the trap were capped and the traps were placed vertically in the ground until entrance holes were flush with the soil surface. The trap interior was coated with an approximate 20:1 mixture of vaseline and baby-oil (Johnson and Johnson, Skillman, NJ) to prevent weevil escape. Although Fluon® (E.I. duPont de Nemours, Wilmington, DE) is commonly recommended for use in coating trap interiors (Rieske and Raffa 1990, 1991), we have found it difficult to obtain commercially and unreliable in preventing weevil escape.

Synthetic baits were dispensed from 25 ml glass vials and were suspended 3 cm below ground level on a 14 gauge aluminum wire threaded through two entrance holes in the trap wall. Three different experiments were conducted during the summer of 1995 in an unforested meadow in order to reduce the presence of competitive host odors to which the weevils might respond. In the first two experiments, baits consisted of different volumetric ratios of 95% ethanol and turpentine mixed together and dispensed from one vial. The turpentine was Klean Strip Product # SD-81 (Div. W.M. Barr Inc., Memphis, TN). The constituents of this product, as determined by GC-MS analysis, are listed in Table 5.

The pit traps were a modified version of those used by Doggett *et al.* (1977). Freshly-cut *P. strobus* bolts, 8 to 12 cm in diameter and 30 cm long, were placed in a shallow pit. The bolts were treated with Lindane® in order to prevent weevil escape. The traps were partially covered with a black tile and fresh *P. strobus* slash to reduce desiccation. A 5:1 ethanol-turpentine bait was dispensed from 25 ml glass vials.

Mark-and-Recapture

Weevils used in this mark-and-recapture study came from a laboratory colony of *H. pales* continuously reared from wild adults collected in local Christmas tree plantations using the method of Speers and Cody (1975). They were stored in clear plastic boxes (22.5 X 30.5 cm) at 24° C (RH: 52%; 14:10 L:D), and fed *P. strobus* billets. Wild weevils were added biweekly to the colony in spring and summer to maintain genetic diversity. They were sexed and scrutinized for the presence of all appendages, and for absence of mites prior to selection. Weevils were starved in individual 50 ml plastic vials containing vermiculite at 24° C for 24 h (RH: 52%; 14:10 L:D) prior to release.

Marking was performed on nonanesthetized individuals during daylight

Table 5. Constituents found in Klean Strip Turpentine (PD# SD-81), as determined by GC-MS analysis, used in mark-and-recapture studies to assess the efficacy of various attractants and trap designs in capturing *H. pales* adults.

Compound	**Percentage of total**
α- pinene	62.1
camphene	20.1
β-pinene	10.8
limonene	2.6
benzene	1.8
3-carene	1.3
cyclohexane	0.8
myrcene	0.2
α-phellandrene	0.1
unknown	0.2

hours, the period of minimal weevil activity (Corneil and Wilson 1980). Acrylic paint pens (Speedball®, Hunt Manufacturing Co., Statesville, NC) were used to mark the upper right elytra of each insect. Separate colors were used for each plot. Preliminary studies showed this method had no adverse effects on weevil activity or survival. A laboratory bioassay to measure the response of marked and unmarked individuals to an attractive bait showed both groups responded similarly ($F_{1,16} = 0.41$; $P = 0.53$). Marked individuals were released at dusk, the period when weevil activity begins to increase (Corneil and Wilson 1980).

The experimental design was a randomized incomplete block with plots serving as blocks (Hicks 1982). Six plots were sampled in each of three separate experiments. Plots were denoted by the presence of a wooden stake indicating the center of each plot. Four traps were randomly assigned in each of the six plots among trap treatments for each of the three experiments. The treatments were evaluated for their effectiveness in capturing *H. pales* adults. Blocks were placed 30 m apart in order to reduce interference with one another. The data for each experiment were square root transformed and analyzed using PROC GLM and the Tukey test for mean separations (SAS Institute 1989).

Experiment 1: *PVC-pit trap test*

Eight treatments (3 reps/treatment) were examined in this experiment. Four PVC pitfall traps were baited with ethanol-turpentine ratios of 1:5, 2:1, 5:1, and ethanol alone. The release rates were determined under laboratory conditions to be 431.7, 594.7, 628.6, and 566.8 mg/24 h at 24.5 ° C (RH=50.9%) respectively. In addition, a fifth unbaited PVC pitfall trap was tested. The three pit trap treatments tested were: 1) unbaited, 2) pine billet only, and 3) pine billet and a synthetic bait of 5:1 ethanol-turpentine at the previously mentioned release rate.

The treatments were randomly placed 5 m from each plot center. Sixty marked individuals (30 males, 30 females) were released from each plot center at dusk on 20 April. Traps were checked every three days for a period of 28 d.

Experiment 2: *Synthetic-natural host material bait test*

Six treatments (4 reps/treatment) were examined for their effectiveness in catching *H. pales*. From the observations of Experiment 1, it was obvious that the natural pine material incorporated in pit traps was much more effective in attracting weevils. Using this information, we chose to test a PVC pitfall trap containing a *P. strobus* billet, 5 cm in diameter and 12.5 cm in length. Two additional PVC pitfall traps containing a 5:1 ethanol-turpentine mixture of varied release rates were tested. The release rates were determined to be 628.6 and 1304.6 mg/24 h at 24.5 ° C (RH=50.9%). Two other PVC pitfall traps were baited with a 2:1 ethanol-turpentine ratio of varied release rates. The release rates were determined to be 594.7 and 1352.1 mg/24 h at 24.5 ° C (RH=50.9%).

A pit trap containing a pine billet and a synthetic bait of 5:1 ethanol-turpentine (release rate: 628.3 mg/ 24 h at 24.5 ° C, RH=50.9%) was also tested.

The treatments were randomly placed 3.5 m from the plot center, in a similar configuration as in Experiment 1. Fifty marked individuals (25 males, 25 females) were released from each plot center at dusk on 23 May. Traps were checked every four days for a period of 24 d.

Experiment 3: *Turpentine constituent test*

Numerous monoterpenes are present in the oleoresin of a coniferous host, and their proportions vary widely among hosts (Mirov 1961). The degree of insect attraction varies considerably with the ratio between compounds, and can begin to exert repellency at high concentrations (Visser 1986). Considering this information and the results observed in Experiments 1 and 2, we attempted to simulate the proportions of each monoterpene constituent found in the oleoresin of *P. strobus*, the preferred host of *H. pales* in local Christmas tree plantations. Mirov (1961) reported a 8.3:1.7 ratio of α-pinene to β-pinene present in the oleoresin of *P. strobus*. These ratios are close to those observed in the turpentine (8.5:1.5), but warranted further investigation.

Eight treatments (3 reps/treatment) were tested in this experiment. Seven PVC pitfall trap treatments were tested: 1) pine billet only, 2) pine billet and 5:1 ethanol-turpentine attractant (release rate: 628.3 mg/24 h at 24.5 ° C, RH=50.9%), 3) 5:1 ethanol-turpentine attractant (release rate: 628.3 mg/24 h at 24.5 ° C, RH=50.9%), 4) 2:1 ethanol-turpentine attractant (release rate: 594.73 mg/24 h at 24.5 °C, RH=50.9%), 5) α-pinene (release rate: 96.8 mg/24 h at 24.5 ° C, RH=50.9%), 6) 7.5:1.5:1 (v/v) α-pinene, β-pinene, and ethanol (release rate: 416.75 mg/24 h at 24.5 ° C, RH=50.9%), and 7) 5:1 ethanol and simulated *P. strobus* oleoresin (8.3:1.7 α-pinene and β-pinene ratio) (release rate: 595.5 mg/24 h at 24.5 ° C, RH=50.9%). A pit trap containing natural host material and the 5:1 ethanol-turpentine attractant at the release rate given above was also assayed.

The treatments were randomly placed 3.5 m from the plot center. Forty marked individuals (20 males, 20 females) were released from each plot center at dusk on 19 July. Traps were examined every three days for a period of 32 d.

RESULTS

Experiment 1: *PVC-pit trap test*

The overall recovery rate of *H. pales* was 27% (n=97), with the majority of weevils being captured in the last two weeks of the sampling period. The treatment effect was significant for both sexes of *H. pales* ($F_{7,459} = 13.26$; $P < 0.0001$). No significant interactions were observed ($F_{7,459} = 0.40$; $P = 0.90$). Both sexes responded similarly in attraction to the treatments ($F_{1,459} = 1.00$; $P = 0.32$), therefore the data were pooled. Pit trap treatments caught significantly more weevils than PVC pitfall trap treatments (Table 6). The pit trap treatment baited with natural host material and a synthetic 5:1 ethanol-turpentine attractant was most effective in trapping weevils (Table 6), accounting for 60% (n=58) of the total weevils recaptured. The number of *H. pales* caught in PVC pitfall traps did not differ significantly from those caught in unbaited traps (Table 6).

Experiment 2: *Synthetic-natural host material bait test*

The recovery rate of *H. pales* was 36% (n=108), with the majority of weevils being captured in the first few weeks following the release of marked individuals. The treatment effect was significant for both sexes of *H. pales* ($F_{5, 271} = 28.44$; $P < 0.0001$). No significant interactions were observed ($F_{5, 271} = 1.21$; $P = 0.32$). Both sexes responded similarly in their attraction to the treatments ($F_{1, 271} = 0.27$; $P = 0.60$), therefore the data were pooled. Pit traps baited with natural pine material and a synthetic 5:1 ethanol-turpentine attractant were most effective in capturing weevils (Table 7), accounting for 62% (n=67) of the total weevils recaptured. PVC pitfall traps baited with a pine billet caught 36% (n=39) of the weevils (Table 7). Synthetically-baited PVC pitfall traps caught only two weevils and their means were not significantly different (Table 7).

Table 6. Mean number (± S.E.) of *H. pales* adults caught in Experiment 1, using mark-and-recapture techniques to assess trap efficacy.

Treatment	N	$\bar{x}$ ± S.E. [a]
PIT (pine & 5:1 attractant)	60	0.97 ± 0.33 a
PIT (pine only)	60	0.55 ± 0.25 b
PVC (1:5 attractant)	60	0.05 ± 0.04 c
PVC (2:1 attractant)	60	0.03 ± 0.03 c
PVC (5:1 attractant)	60	0.02 ± 0.02 c
PVC (ETOH attractant)	60	0.00 ± 0.00 c
PIT (unbaited)	60	0.00 ± 0.00 c
PVC (unbaited)	60	0.00 ± 0.00 c

[a] Means followed by the same letter are not significantly different (P > 0.05; Tukey test).

Table 7. Mean number (± S.E.) of *H. pales* adults caught in Experiment 2, using mark-and-recapture techniques to assess trap efficacy.

Treatment	**N**	**$\bar{x}$ ± S.E.**[a]
PIT (pine & 5:1 attractant)	48	1.40 ± 0.35 a
PVC (pine only)	48	0.81 ± 0.24 b
PVC (2:1 attractant; 594.7 mg/24 h)	48	0.02 ± 0.02 c
PVC (5:1 attractant; 628.3 mg/24 h)	48	0.02 ± 0.02 c
PVC (5:1 attractant; 1304.6 mg/24 h)	48	0.00 ± 0.00 c
PVC (2:1 attractant; 1352.1 mg/24 h)	48	0.00 ± 0.00 c

[a] Means followed by the same letter are not significantly different ($P > 0.05$; Tukey test).

Experiment 3: *Turpentine constituent test*

The overall recovery rate of *H. pales* was 18% (n=43), with all weevils being captured in the first two weeks following the release of marked individuals. Repeated severe rainstorms may have contributed to the reduced catch after the initial two weeks. The treatment effect was significant ($F_{7,411}$ = 3.34; P < 0.002). No significant interactions were observed ($F_{7,411}$ = 0.29; P = 0.96). Both sexes responded similarly to the treatments ($F_{1,411}$ = 0.62; P = 0.43), therefore the data were pooled. Traps baited with natural host material were more effective than those with synthetic baits (Table 8). Pit traps containing natural host material and a 5:1 ethanol-turpentine mixture were most effective in capturing weevils (Table 8), accounting for 43% (n=19) of the total weevils recaptured. PVC pitfall traps baited with pine, and pine and a synthetic 5:1 ethanol-turpentine bait did not differ significantly from the similarly baited pit trap. PVC pitfall traps baited with synthetic baits attempting to imitate the monoterpene composition of *P. strobus* oleoresin were not effective in capturing weevils (Table 8).

DISCUSSION

In this study, *H. pales* adults were most attracted to the presence of natural host material. The trap type containing this pine material seemed to have little effect on the overall attractiveness of the trapping system. These

Table 8. Mean number (± S.E.) of *H. pales* adults caught in Experiment 3, using mark-and-recapture techniques to assess trap efficacy.

Treatment [a]	**N**	$\bar{x}$ **± S.E.** [b]
PIT (pine & 5:1 bait)	54	0.35 ± 0.21 a
PVC (pine only)	54	0.26 ± 0.16 ab
PVC (pine & 5:1 bait)	54	0.13 ± 0.09 ab
PVC (5:1 bait)	54	0.04 ± 0.03 ab
PVC (5:1 simulated oleoresin bait)	54	0.02 ± 0.02 b
PVC (7.5:1.5:1 bait)	54	0.02 ± 0.02 b
PVC (α-pinene bait)	54	0.00 ± 0.00 b
PVC (2:1 bait)	54	0.00 ± 0.00 b

[a] 2-way ratios are ethanol-turpentine; 3-way ratios are α–pinene, β-pinene, and ETOH

[b] Means followed by the same letter are not significantly different (P > 0.05; Tukey test).

data provide an explanation for why pit traps baited with natural host material and synthetic volatiles were more attractive than PVC pitfall traps baited with synthetic volatiles only (Chapter 2). The absence of attraction to unbaited traps supports data from Raffa and Hunt (1988) that PVC pitfall traps do not provide any visual or physical attraction to weevils in the absence of volatiles.

Previous studies have reported a 1:1 sex ratio using baited pitfall traps (Raffa and Hunt 1988, Hunt and Raffa 1989, Rieske 1990, Rieske and Raffa 1991). The lack of significant differences in recapture rates between male and female *H. pales* provides further evidence that these volatiles are not simply ovipositional cues, but may act as both feeding and mating cues. Mating may occur as a result of chance encounters between males arriving to commence feeding activities and females searching for feeding or ovipositional habitats.

In Experiment 3, we attempted to simulate the ratio of α-pinene and β-pinene components found in the oleoresin of *P. strobus,* testing the hypothesis that this simulation would be as attractive to local populations of *H. pales* as the natural host material. Adult weevils may cue heavily into the ratio between constituents, resulting in their repeated selection of natural host material baits. Our results suggest that some volatiles emanating from *P. strobus* billets that were not included in this bait simulation may be important in attracting weevils.

The PVC pitfall trap baited with synthetic attractants has been shown to be effective in monitoring weevil populations in the Lake States and Europe (Nordlander 1987, Raffa and Hunt 1988, Hunt and Raffa 1989, Rieske 1990, Rieske and Raffa 1991, 1993). In western Virginia, attempts to incorporate this trapping technique into sampling programs have been unsuccessful (Chapter 2). In this study, PVC pitfall traps baited with synthetic baits of ethanol and turpentine caught few weevils relative to traps containing natural host material. Laboratory assays have shown that ethanol-turpentine baits are attractive to *H. pales* (Chapter 4), but few differences in attraction among ratios were observed. However, when these baits are placed in the field they seem to lose attractiveness or are outcompeted by natural host odors. In Experiment 2, PVC pitfall traps baited with increased release rates of ethanol and turpentine did not attract weevils, suggesting the observed decrease in attraction does not result from competition with natural host odors. The use of a PVC pitfall trapping system would be quite desirable as these traps are less costly and labor intensive to implement than the aforementioned pit traps. However, our data suggests we must continue to rely on the use of pit traps containing natural host material to monitor weevil populations in Virginia, until we can develop an appropriate, easily implemented bait system for the PVC pitfall trap.

CHAPTER 4

Attraction of *Hylobius pales* (Herbst) to Varying Levels of Ethanol and Turpentine in a Laboratory Bioassay

INTRODUCTION

Olfactory receptor systems enable insects to perceive signals and compile odor cues produced by a host that act as a chemical message (Visser 1986). Insect species often feed on a restricted range of plants, partly because plant odors are highly specific and comprised of compounds not found in unrelated species (Visser 1986). Terpenes and associated derivatives that are present in conifer resins attract a number of insect species. Volatile monoterpenes, released from stressed or wounded conifers, are known to attract *Hylobius* spp. (Thomas and Hertel 1969, Mustaparta 1975, Tilles *et al.* 1986a). There are many different monoterpenes present in the oleoresin of coniferous hosts that are largely genetically determined (Visser 1986). Their proportions vary widely among tree species, individual trees, and different tissues within the same tree. They are thought to provide both feeding and ovipositional cues in *H. pales,* as there is an absence of any known long range sex pheromone (Wilson and Millers 1983). Both ethanol and turpentine are reported as simulated host cues for *Hylobius* weevils (Tilles *et al.* 1986a, Raffa and Hunt 1988, Hunt and Raffa 1989). Their synergistic effect make baits more attractive (Raffa and Hunt 1988, Hunt and Raffa 1989). However, the degree of attraction varies with the ratio between compounds, and can exert repellency at high concentrations (Visser 1986).

Turpentine is comprised primarily of host monoterpenes of which α-pinene and β-pinene are the major constituents. The primary attractant of conifer feeding *Hylobius* spp. is α-pinene (Chenier and Philogene 1989), although electrophysiological reception studies show *Hylobius abietis* L. is attracted also to β-pinene (Mustaparta 1975). Ethanol is a product of anaerobic plant and microbial metabolism and levels are known to vary considerably among species and seasons (Crawford and Baines 1977). Ethanol levels tend to rise with increased plant stress, and usually peak in April and May (Kimmerer and Kozlowski 1982). Monoterpenes are believed to provide information on host species identification, while ethanol may signify the plant is under stress (Rieske and Raffa 1991).

Ethanol and turpentine-baited pitfall traps have been used for monitoring *Hylobius* weevil populations (Tilles *et al.* 1986a, b, Nordlander 1987, Raffa and Hunt 1988, Hunt and Raffa 1989, Rieske and Raffa 1991, 1993). Previous studies have shown a 1:1 volumetric ratio of ethanol and turpentine will attract weevils whereas unbaited traps, or traps with either compound alone, show no attraction (Raffa and Hunt 1988, Hunt and Raffa 1989). Rieske and Raffa (1991) found *H. pales* are most attracted to ethanol-turpentine ratios

≥ 5:1 in field studies conducted in Wisconsin Christmas tree plantations. Traps baited with decreasing levels of ethanol were less attractive. Both males and females responded similarly in their attraction to these compounds. However, they suggest weevil attraction to baits may vary with respect to gender, age, or reproductive state.

Field assay procedures, although necessary at some stage in the evaluation of a compound, have inherent disadvantages. Variations in temperature, rainfall, competitive host odors, and insect distributions restrict the effectiveness of field assays. Laboratory assays allow the investigator to test material in a controlled, constant environment. In this study, behavioral bioassays were performed to determine the attraction of simulated host odors containing varying ethanol-turpentine ratios to walking weevils. Weevils from three age classes, two pre-reproductive and one post-reproductive, were assayed for each of 8 treatments.

MATERIALS AND METHODS

Assay Apparatus

The assay apparatus was constructed of Plexiglas™ and modified after the open-arena, pedestrian design described by Wood and Bushing (1963), and modified by Payne *et al.* (1976). The arena surface measured 22.5 x 27 cm, and accommodated a sheet of photocopy paper (Hammermill Papers™) which was replaced after each assay. Airflow was supplied by bottled medical breathing air, and delivered to the arena surface at a 1 l/min airflow rate through a 7 mm dia. plastic tube. The airflow was filtered through a Drierite anhydrous $CaSO_4$ desiccant and charcoal filter (W.A. Hammond Drierite Co., Xenia, Ohio). Odor stimuli were eluted at the end of the air flow outlet and were removed from the assay room through an exhaust fan at the distal end of the arena stage.

Elution Device

Solutions were eluted into the airstream at 1 µl/min from a 10 µl, 700 series Hamilton syringe (Hamilton Co., Reno, NV). The syringe was constantly depressed by a rack and pinion gear driven elution device and powered by a 30 min., spring driven timer motor mounted on a 3 mm thick aluminum plate (16.5 x 30.5 cm) attached to a 2.5 cm angle iron base. The brass pinion gear (5 cm dia., 3 mm pitch; Boston Gear Y64128) was threaded to the shaft motor (Payne *et al.* 1976). At the top of the aluminum plate was a slide base that made contact with the pinion gear. This component could be manipulated to provide contact with the end of the plunger at the initiation of assay replicates. The syringe was held in position on the aluminum plate by a plastic clip attached at the anterior end of the elution device.

Assay Room

To provide a controlled environment, the assay apparatus was enclosed by a wooden frame covered with aluminum screening and black plastic. The temperature of the assay room was maintained at 20.5 ° C. The room was kept dark by the drawing of shades on the three windows. To allow observations and manipulations of equipment during replicates without disturbing the test insects, the assay stage was fitted with a red lamp (Phillips and Burkholder 1981). Chemicals were stored in a fume hood to reduce the possibility of contamination.

Assay Procedure

Weevils used in this study were obtained from a laboratory colony of *H. pales* continuously reared on *Pinus strobus* (L.) billets using the techniques developed by Speers and Cody (1975), with biweekly infusions of wild adults collected from local Christmas tree plantations during spring and summer. Weevils were sexed and scrutinized for the presence of all appendages, and for absence of mites prior to selection for the bioassay. Weevils used in the bioassay were placed in individual 50 ml plastic vials containing vermiculite and starved at 24° C for 24 h (RH: 52%; 14:10 h L:D) prior to the initiation of bioassay procedures. Assay solutions were stored in a deep freeze at -60° C prior to assay tests.

For each assay, 5 weevils of known age and sex were placed onto the arena stage, 9 cm from the volatile release point. Those weevils that came within 1 cm of the release point were counted for positive response. The assay was continued for 8 minutes or until all weevils had elicited a positive response. Due to a limited supply of weevils in each age class, they sometimes were subjected to more than one assay in a given day. Assayed weevils were rested for at least 1.25 h prior to being subjected to another test. This technique was determined to be acceptable for the pine engraver beetle, *Ips pini* (Say), by Hager and Teale (1994). Preliminary studies to determine optimal test concentrations, in which weevils were assayed only once, resulted in a response similar to those in which weevils were subjected to multiple assays in a given day (73.3% response vs. 73.1% response). After each assay, the stage, syringe, and air flow outlet were cleaned with pentane to avoid contamination between replicates. A new paper surface was then placed on the arena stage.

Seven attractants and a standard of pentane were assayed for at least 5 weevils of both sexes within an age class for each day. Treatments included ethanol and turpentine ratios of 1:5, 1:1, 2:1, 5:1, 10:1, and single applications of ethanol, turpentine, and pentane. The turpentine was Klean Strip Product # SD-81 (Div W.M. Barr Inc., Memphis, TN). The constituents of this product, as determined by GC-MS analysis, are listed in Table 5. All treatments were tested at a concentration of 1 μg/ml, using pentane as the solvent. The solvent was tested to measure the relative activity level of the weevils and as an indicator of possible contamination.

Weevils from three age classes (2 wk, 6 wk, and 20 wk) were assayed for each of the 8 treatments. Fifty males and 50 females were assayed for each treatment per age class, for a total of 2400 weevils in the experiment. An arcsine square root transformation was performed on the data. The data were analyzed with an analysis of variance ($\alpha = .05$) using PROC GLM and the Tukey test for mean separations (SAS Institute 1989).

RESULTS

There was a significant age x treatment interaction ($F_{14, 427} = 2.01$; $P < 0.01$), although no other interactions were found to be significant. Therefore, the data were further analyzed for age. Blocking was not an effective means of explaining the variation between weevil responses. Both sexes responded similarly to the treatments, therefore the data were pooled, and the results pertain to both sexes in each experiment.

Few significant differences among treatment means were observed for each age class. Two week old *H. pales* were less attracted to turpentine and pentane than to the rest of the treatments (Figure 9). The treatment effects were not significant for sex ($F_{1, 139} = 0.30$; $P = 0.58$). Six week old weevils were most attracted to the 5:1 ethanol-turpentine ratio, although responses to this treatment were only significantly greater than the standard of pentane (Figure 9). The treatment effects were not significant for sex ($F_{1, 139} = 0.18$; $P = 0.68$). Twenty week old *H. pales* were equally attracted to treatments containing

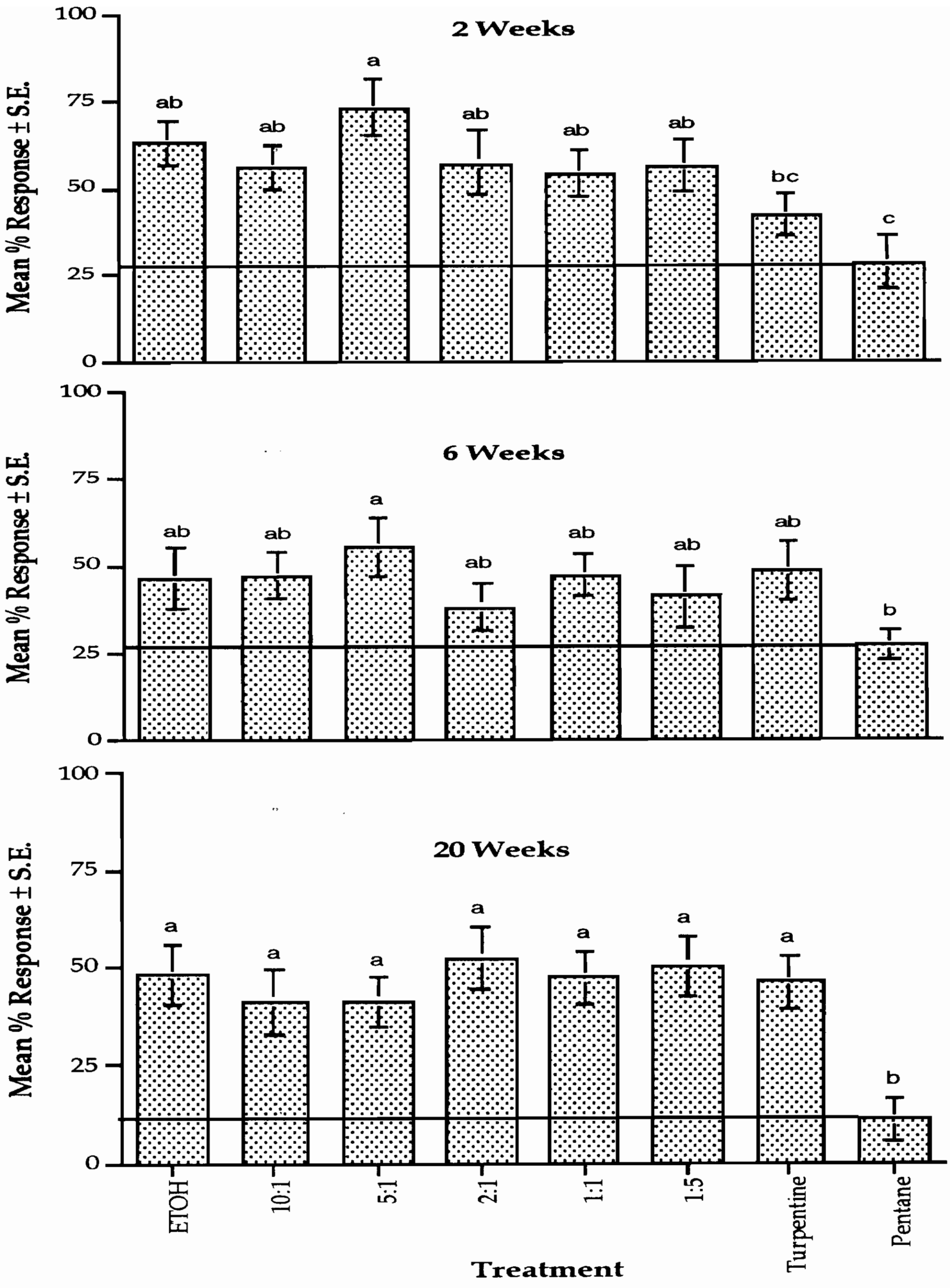

Figure 9. Mean percent response (± S.E.) of *H. pales* adults to different ethanol-and-turpentine ratios in a laboratory bioassay. Means followed by the same letter within an age group are not significantly different (P > 0.05; Tukey test).

ethanol and turpentine ratios, ethanol, and turpentine (Figure 9), and responses were significant greater than those to pentane. The treatment effects for sex were not significant ($F_{1,141} = 1.29$; $P = 0.26$).

DISCUSSION

Hylobius pales males and females responded very similarly in their attraction to the test treatments. These equivalent responses further suggest that these volatiles must function as both feeding and ovipositional stimulants. Rieske and Raffa (1991) found both sexes responded similarly to baits in field assays conducted in Wisconsin Christmas tree plantations. They suggest that these volatiles may also facilitate mating location, since there is an absence of any known long range sex pheromone. Male attraction to baits is not synchronous with gonad development (Hoffman unpublished data), although, mating pairs are often observed at the base of host trees (Wilson and Miller 1983). Perhaps mating occurs as a result of chance encounters between males arriving to commence feeding activities, and females present through reception of both feeding and ovipositional cues.

It has been suggested that variation may occur in weevil attraction to baits with respect to age, seasons, and gender (Hunt and Raffa 1989, Rieske and Raffa 1991, 1993). Since both age and seasonality are closely related to reproductive readiness, this may be the underlying factor influencing differences observed among age classes. Hoffman and Raffa (1992) found 85%

of male *H. pales* passed sperm to receptive females at 4 weeks of age. This suggests that *H. pales* adults, at least males, begin completing sexual maturation at this age. Clearly, 6 weeks of age is in close proximity of attaining sexual maturation. Our results suggest variations in weevil response do not exist among age, gender, or reproductive state. Apparent trends in weevil response were observed, however, they were not supported by statistical differences. It seems sexually immature weevils may be more attracted to baits containing increased levels of ethanol, while sexually mature weevils may respond at increased rates to baits containing decreased levels of ethanol. Further studies are needed to determine if such a trend actually exists. Although the sample size of this study was quite large, further replication would reduce experimental error, increasing the precision of treatment mean estimates.

SUMMARY

1) It was shown that natural-host baited pit traps were more effective in capturing *H. pales* and *P. nemorensis* than synthetically-baited PVC pitfall traps.

2) Trap rotation and vegetation management practices had no effect on trap catch.

3) *Hylobius pales* and *P. nemorensis* had similar seasonal distributions, with both varying similarly between the two years during which this study was conducted.

4) No correlation was established between trap catch and weevil-induced seedling damage within or among sample periods, or between years.

5) Vegetation management practices did affect weevil access to target seedlings. The amount and density of secondary ground vegetation corresponded directly with observed seedling damage.

6) *Hylobius pales* adults were most attracted to traps baited with natural host material as determined by mark-and-recapture techniques. The trap type containing this material seemed to have little effect on the overall attractiveness of the trapping system.

7) A laboratory bioassay was used to determine that variations in weevil response do not exist among gender, age or reproductive state.

8) Both sexes responded similarly to all treatments in these studies.

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VITA

Christopher John Fettig was born on November 3, 1970 in Queens, New York. He graduated from Raritan High School in Hazlet, New Jersey in June, 1989. Following high school, Mr. Fettig attended Virginia Tech in 1989. He graduated in May 1993 with a BS degree in Forestry, and began a MS program in Entomology in August 1993 under the supervision of Dr. Scott M. Salom. His thesis was defended on February 23, 1996.