

BIOLOGY AND CONTROL OF THE WESTERN POPLAR CLEARWING MOTH,
PARANTHRENE ROBINIAE (HY. EDWARDS), IN HYBRID POPLARS.

By
NEAL THOMAS KITTELSON

This dissertation is submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

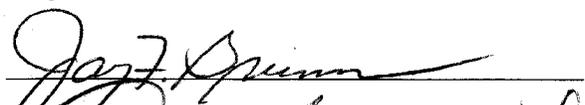
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December 2006

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of
NEAL THOMAS KITTELSON find it satisfactory and recommend that it be accepted.


Chair




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PARANTHRENE ROBINIAE (HY. EDWARDS), IN HYBRID POPLARS.

Abstract:

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Chair: John J Brown

Western poplar clearwing moths [*Paranthrene robiniae* (Hy. Edwards)] are endemic in Pacific Northwest riparian habitats at low population densities. These moths have colonized commercial hybrid poplar plantings where larvae damage the poplar trees by boring into the trunks. In newly planted poplars a single *P. robiniae* larva can cause tree mortality. Prior to the introduction of agroforestry into eastern Washington and Oregon *Paranthrene robiniae* (Hy. Edwards) was not considered a pest of economic importance.

In 2002, there was an outbreak of *P. robiniae*, documented with pheromone baited trap catches increasing 190-fold from those of 2001. Chlorpyrifos applications failed to reduce *P. robiniae* populations. Investigatory research started in August 2002, to see if a pheromone control strategy would be feasible. In this preliminary study we found that we were able to shut down trap capture of *P. robiniae* in 0.4 ha plots by attaching pheromone lures to every other tree.

We began a pheromone control strategy in 2003, but because of difficulties in acquiring adequate pheromone, and late applications, we were unable to show a reduction in damage to first and second yr trees. We continued our pheromone control strategy in 2004 with adequate pheromone to cover entire farms and applied the pheromone before *P. robiniae* began their flight season. With that approach, we were able to show a

reduction in damage to first and second yr trees. With sustained use of this pheromone control strategy we have continued to document reductions in damage.

With persistent pheromone saturation, trap capture of *P. robiniae* was shut down to the point where weekly counts of the monitoring traps did not provide an adequate way to monitor *P. robiniae* populations, and thus infield pheromone levels. *Nemapogon variatella* (Clemens), a fungus moth, is attracted to the same pheromone as *P. robiniae*. However, we have no evidence to show that *N. variatella* populations have been affected by our pheromone control strategy. By monitoring *N. variatella* in monitoring traps baited with *P. robiniae* pheromone, we can use *N. variatella* as a “canary” species and monitor ambient in field pheromone levels in the absence of *P. robiniae*.

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Dedication

To Mindy Sue, my motivation.

To my parents, Rick & Gretchen, for all their support.

Chapter 1: Introduction to *Paranthrene robiniae* (Hy. Edwards), the western poplar clearwing moth.

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Prior to the introduction of agroforestry into eastern Washington and Oregon *Paranthrene robiniae* (Hy. Edwards) was not considered a pest of economic importance, therefore there is a lack of information on the life history and biology of *P. robiniae* in the literature. The first recorded capture of *P. robiniae* in the M. James Insect Collection at Washington State University, Pullman WA was not until 1951. Historically, *P. robiniae* was reported only to be a pest of ornamentals and in some nursery stock (Bently et al. 1994). Bently et al. (1994) found that an application of chlorpyrifos provided adequate control in these situations. However, when an outbreak of *P. robiniae* occurred in 2002 in commercial poplar plantations managed by Potlatch Corporation (Spokane, WA) and Boise Cascade (Boise, ID), repeated chlorpyrifos applications failed to provide adequate control (Brown et al. 2006).

Current classification. *Paranthrene robiniae* was originally described as *Sciapteron robiniae* by Henry Edwards in 1880 (Eichlin and Duckworth, 1988); since then it has been reclassified into the *Paranthrene* genus. The current classification is as follows:

Order: Lepidoptera

Superfamily: Sesiioidea

Family: Sesiidae

Subfamily: Paranthreninae

Tribe: Paranthrenini

Genus: *Paranthrene*

Species: *robiniae* (Hy. Edwards)

General biology. *Paranthrene robiniae* is a brightly colored diurnal moth belonging to the family Sesiidae (Fig. 1). Almost all members of the sesiid mimic bees and wasps in appearance and behavior (Eichlin and Duckworth, 1988). Male and female *P. robiniae* have slight sexually dimorphic characters as pupae and adults. The most notable adult feature is the antennal difference between females (Fig. 2A) and males (Fig. 2B), which conforms to the general description of sesiids by Eichlin and Duckworth (1988). Both male and female have clavate antennae, however, the male's antennae are ciliate on the ventral side. As with the dogwood borer (*Synanthedon scitula* (Harris)) (Leskey and Bergh, 2003), *P. robiniae* male and female pupae have a different number of fused terminal abdominal segments with single rows of spines, which can be used to determine sex. Female *P. robiniae* have a single row of spines on four fused abdominal segments (7-10) (Fig. 3), while males have a single row of spines on three fused abdominal segments (8-10) (Fig. 4). Larvae of sesiid moths are borers, in the stems, roots, branches, and trunks of herbaceous plants (Eichlin and Duckworth, 1988) (Fig. 5). *Paranthrene robiniae* eggs fit the general description of sesiid eggs given by Eichlin and Duckworth (1988); "...brown, ovate and slightly concave dorsally and ventrally, with the surface usually sculptured with minute shagreening in hexagonal designs." (Fig. 6).

Biological Control. We have found a suspected braconid parasitoid of *P. robiniae* (Fig. 7), but it is still to be identified. Specimens were sent to James Whitfield at the University of Illinois at Urbana-Champaign, Illinois to be identified. Voucher specimens were deposited in the M. James Insect Collection at Washington State University. Late instar larvae collected from the field were brought into the laboratory for observation. Several of these larvae appeared to be parasitized; they were a pale

white color, which is different from the normal cream color. After several days in the laboratory, numerous parasitoids emerged from the larva and pupated inside individual cocoons on the host's body. Approximately five days later adult parasitoids began to emerge. We suspect the parasitoid is *Apanteles paranthrenidis* (Muesebeck) (Hymenoptera: Braconidae) because this species has been identified as a parasitoid of *P. robiniae* (Krombein et al. 1979). From what we can tell, the suspected parasitoid is similar to the braconid parasitoid found by Georgiev (2001a) in *Paranthrene tabaniformis* (Rottemburg) in eastern Europe.

Literature specific to *P. robiniae*. There is little literature about *P. robiniae*. Solomon and Dix (1979) have only one reference to *P. robiniae* (*Sciapteron robiniae*) (Riley and Howard, 1890) in their bibliography of clearwing borers. Kaya and Lindgren (1983) reported biological control of *P. robiniae* through the use of parasitic nematodes. Bentley et al. (1994) described chemical control measures for *P. robiniae*. The female sex pheromone (4:1 (E, Z): (Z, Z)-3, 13-octadecadienyl alcohol) of *P. robiniae* was described by Cowles et al. (1996). Recent reports on pests of hybrid poplar do not even list *P. robiniae* as a pest of poplars (Chastagner and Hudaki 1999, Coyle et al. 2005).

Literature on sessid pests. Despite the lack of information on *P. robiniae* in the literature, other sesiids are important economic pests and have been well described. The peachtree borer *Synanthedon exitiosa* (Say) (Russell and Stanely 1969, Holloway et al. 1977, Barry and Nielsen 1984) and the lesser peachtree borer, *S. pictipes* (Grote and Robinson) are pests in peach orchards (Wong et al. 1969) . The grape root borer, *Vitacea polistiformis* (Harris) is an economically important pest of grapes (Weires 1986, Alm et al. 1989, Webb et al. 1992, Olien et al. 1993). Recently the dogwood borer, *Synanthedon*

scitula (Harris) has become an important pest in apple orchards (Roger and Grant 1991, Pfeiffer and Killian 1999, Eliason and Potter 2000, Bergh and Leskey 2003).

In 2004, we attempted to start a laboratory rearing project for *P. robiniae*. In the literature there have been laboratory colonies established for *S. exitiosa* and *S. pictipes* that would suggest rearing sesiids is not difficult (Cleveland et al. 1968, Antonio et al. 1975).

Rearing methods for *S. scitula* reported by Leskey and Bergh (2002) were more rigorous than those for *S. exitiosa* and *S. pictipes*. Forschler and Nordin (1989) and Moraal (1989) reported techniques for rearing *Paranthrene dollii* (Neumoegen) and *P. tabniformis* (Rottemburg), respectively, which are closely related to *P. robiniae*. Using the methods reported, we successfully reared two *P. robiniae* adults. We had five eggs hatch and only two of those larvae survived to the adult stage. We were able to successfully mate males and females in the laboratory and get females to oviposit, however, of the eggs all but five did not hatch. We could not refine our technique because of an inadequate supply of wild caught specimens, which we attribute to the success of our pheromone control strategy initiated in 2003.

Besides *Synanthedon exitiosa*, *S. pictipes*, *S. scitula* and *Vitecea polistiformis*, research has been conducted on numerous other sesiid pests and their host plants. The majority of the research has been conducted on members of the *Synanthedon* genus: Nielsen et al. (1978) reported *Synanthedon sequoiae* (Hy. Edwards) attacking ornamental *Pinus* spp. and *S. bibionipennis* (Boisduval) attacking strawberries, *Fragaria ananassa* (Weston) Rozier, William et al. (1999) described *S. rileyana* (Hy. Edwards) attacking horsenettle, *Solanum carolinense* (L.), Neal (1984) said *S. rhododendri* (Beutenmüller)

attacks *Rhododendron* spp., Kahounova and Mracek (1991) observed *S. myopaeformis* (Borkhausen) attacking a variety of trees from the Rosaceae family, Anagnostakis et al. (1994) have reported on *S. castaneae* (Busck) attacking American Chestnut [*Castanea dentata* (Marshall) Borkhausen], James et al. (2001) and Grassi et. al (2002) observed *S. tipuliformis* (Clerck) attacking red currants [*Ribes rubrum* L. (Grossulariaceae)], and Rocchini et al. (2003) examined *S. novaroensis* (Hy. Edwards) attacking Douglas fir, *Pseudotsuga menziesii* (Mirbel) Franco. There have been some reports on closely related species to *Paranthrene robiniae*, that are in the same genus: Solomon (1985) observed *P. simulans* (Grote) attacking oak, *Quercus rubra* (L.), Forschler and Nordin (1989) reported on *P. dollii* which attacks trees in the Salicaceae (poplar and willow) family, and Moraal (1989) and Georgiev (2001a,b) reported on *P. tabiniformis* which also attack trees in the Salicaceae family. There are a few other reports of sesiid pests from different genera: Pearson (1995) reported on *Melittia cucurbitae* (Harris) attacking squash, *Cucurbita pepo* (L.), Vickers (2002) observed *Ichneumonoptera chrysophanes* (Meyrick) attacking persimmons, *Diospyros* spp., and Vickers and Rumbo (2002) described *Carmenta chrysophanes* (Meyrick) attacking Australian red ash, *Alphitonia excelsa* (Fenzl) Benth.

My research involved explaining the life history of *P. robiniae* as well as designing and monitoring the implementation of a western poplar clearwing moth pheromone-based control strategy for use in the hybrid poplar system. Although little of the biology for *P. robiniae* has been previously described, the reported biology of other sesiid pests have provided us with some information.

Aspects of the biology of *S. exitiosa* (the peachtree borer) have been described by Russell and Stanley (1969), Smith (1970a), Smith (1970b), Gentry et al. (1972), Yonce (1975), McLaughlin et al. (1976), Holloway et al. (1977), Barry et al. (1978), Tumlinson (1979), Childers et al. (1979), Doolittle et al. (1980), Nielsen and Purrington, 1980, Gentry et al. (1981), Gentry and Wells (1982), Yonce(1982), Barry and Nielsen (1984), Gentry and Snow (1985), Snow et al. (1985), Cossentine et al. (1990), and Brown et al. (1991). Literature on *S. pictipes* (the lesser peachtree borer) biology is also quite extensive (Cleveland et al. 1968, Wong et al. 1969, Smith 1970, Wong et al. 1971, Wong and Cleveland 1972, Yonce et al. 1974, Antonio et al. 1975, Wong and Davis 1975, Yonce 1975, McLaughlin et al. 1976, Yonce et al. 1976, Yonce et al. 1977, Childers et al. 1979, Gentry et al. 1979, Davis et al. 1980, Doolittle et al. 1980, Gentry et al. 1981, Williams et al. 1983, Gentry and Snow 1985, Snow et. al 1985, Anderson et al. 1987, and Reed et al. 1988). More recently, research has focused on the biology of the dogwood borer (*S. scitula*) (Heichel and Turner 1973, Potter and Timmons 1981, Potter and Timmons 1983, Riedl et al. 1985, Walton 1986, Rogers and Grant 1991, Pfeiffer and Killian 1999, Leskey and Bergh 2002, Bergh and Leskey 2003, Bergh et al. 2004, Kain et al. 2004, Zhang et al. 2005, and Bergh et al. 2006).

Pheromone-based control strategies have been reported for the peachtree borer (*S. exitiosa*) (Gentry 1976, Yonce and Gentry 1982, Gentry and Snow 1984, Snow et. al 1985), the lesser peachtree borer (*S. pictipes*) (Wong et al. 1972, Gentry 1976, Gentry and Snow 1984, Snow et. al 1985, Pfeiffer et al. 1991), the grape root borer (*V. polistiformis*) (All et al. 1985, Johnson et al. 1991, Pearson and Meyer 1996) and the currant clearwing moth (*S. tipuliformis*) (James et al. 2001, Grassi et al. 2002, Suckling et al. 2005).

This prior work on the biology and control of various sesiid species, has provided us a foundation on which we started our research. In this dissertation, I have examined and reported on several aspects of the biology and control of *Paranthrene robiniae*:

- 1) Several aspects on the biology and life history of *P. robiniae* that are important for implementation of pheromone control strategies (Chapter 2).
- 2) The documentation of an outbreak of *P. robiniae* and the inadequate control provided by a commercial insecticide (Chapter 3).
- 3) Implementation and monitoring of a successful pheromone control strategy over 4 yrs. (Chapter 4).
- 4) Aspects of pheromone drift related to *P. robiniae*, which may provided economic relief for poplar growers and *Nemapogon variatella*, which may provide an alternative pheromone monitoring strategy (Chpater 5).
- 5) Other sessid species that may be present within the poplar system (Chapter 6).
- 6) A preliminary study on cellulose digestion in *Prionoxystus robiniae* Chapter 7).

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Figures:



Figure 1: Male *P. robiniae* (2003, John J. Brown).

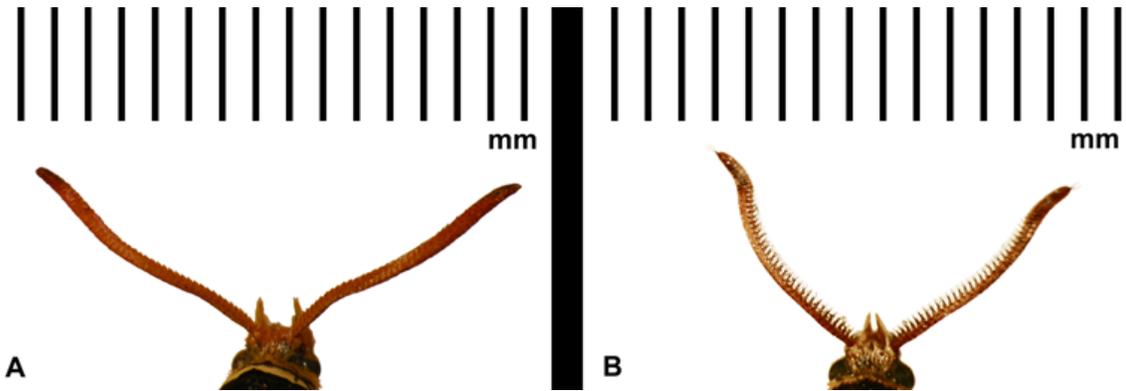


Figure 2: Antennal sexual dimorphism of *P. robiniae* adults. A. Female. B. Male. (2005, Neal T. Kittelson)

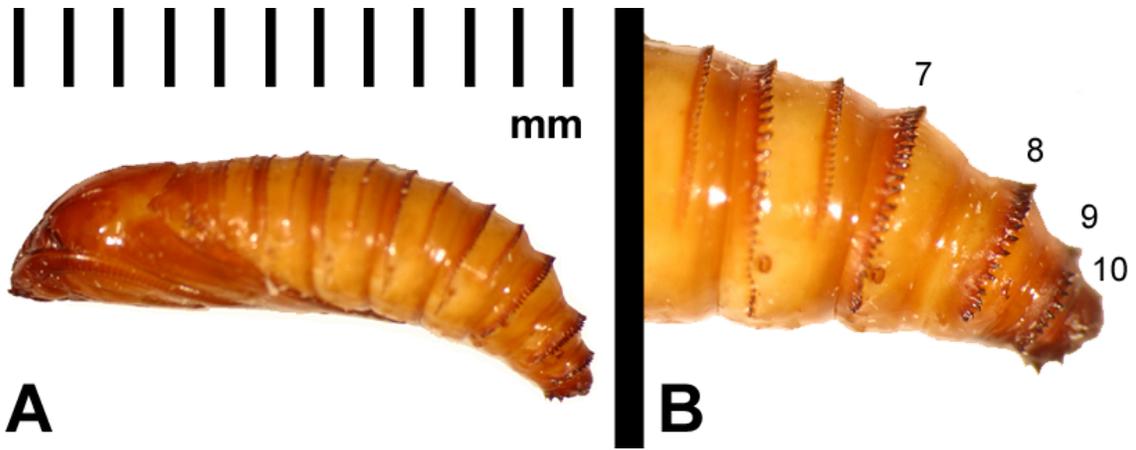


Figure 3: Pupal sexual dimorphism. A: Female pupae. B: Showing single row of spines on the fused 7-10th abdominal segments (2004, Neal T. Kittelson).

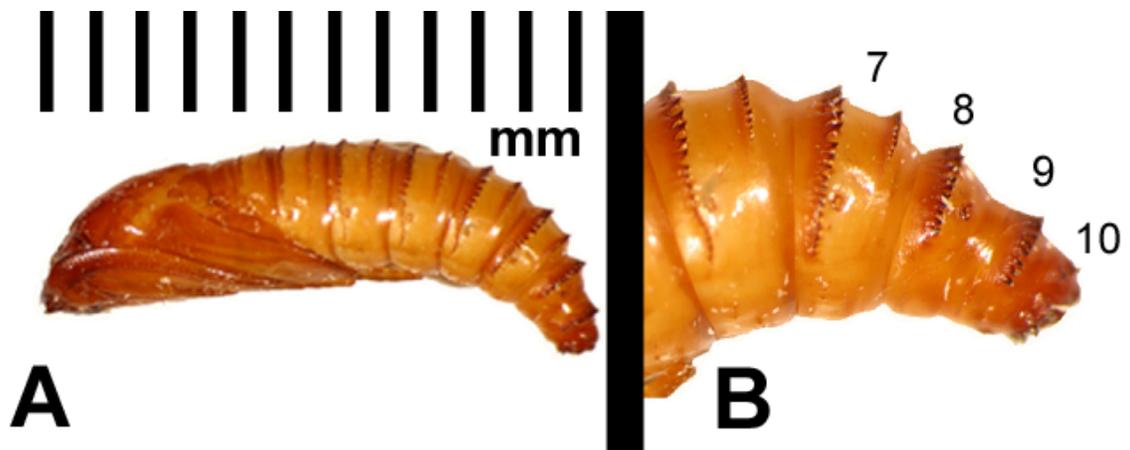


Figure 4: Pupal sexual dimorphism. A: Male pupae. B: Showing single row of spines on the fused 8-10th abdominal segments (2004, Neal T. Kittelson).



Figure 5: Mature *P. robiniae* larva in planting stick (2005, Neal T. Kittelson).

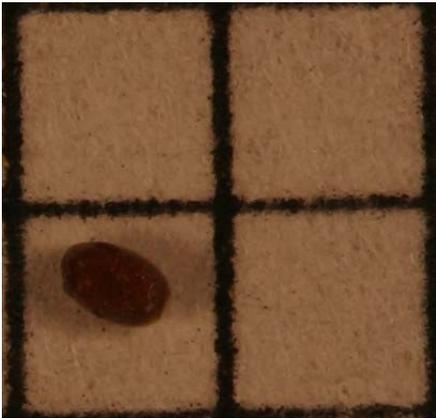


Figure 6: *Paranthrene robiniae* egg. Millimeter grid. (2002, John J. Brown).

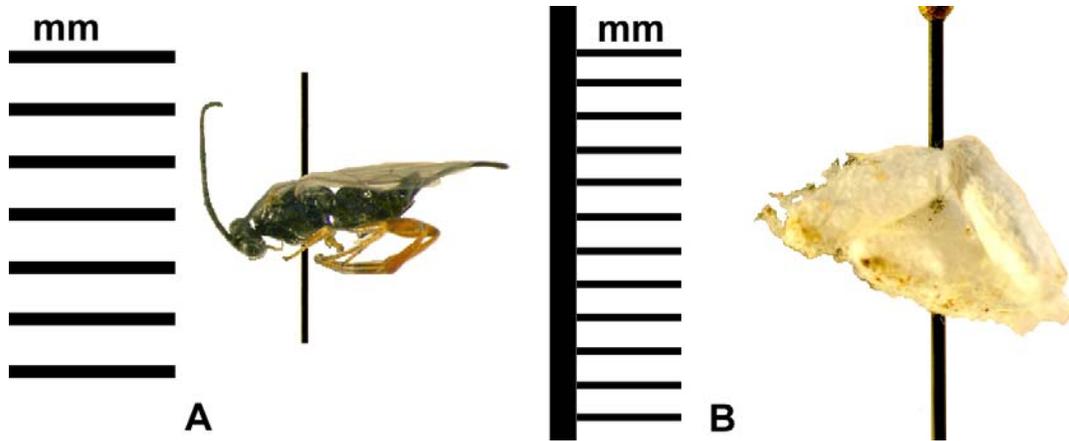


Figure 7: Suspected adult parasitoid (A) of *P. robiniae* and its pupal case (B). (2003, Neal T. Kittelson).

Chapter 2: Biology of the Western Poplar Clearwing Moth [Lepidoptera: Sesiidae],
Paranthrene robiniae.

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ABSTRACT: The western poplar clearwing moth (*Paranthrene robiniae* (Hy. Edwards)) has only recently become an economically important agricultural pest. In northeast Oregon and southeast Washington, poplar plantations are grown for non-structural sawtimber, and galleries of *P. robiniae* cannot be tolerated. Chemical controls have been ineffective against this pest in production situations. Since this is a “new” pest, little of *P. robiniae*’s biology is known. We have five seasons of first male capture data that have allowed us to predict when *P. robiniae*’s mating flight period begins based on degree-day information. We report that although trap color may influence trap catch of this diurnal moth, chemotaxis is a more important cue at long distances. Male *P. robiniae* are strong flyers and very responsive to the female sex pheromone. Tree canopy height may effect where male *P. robiniae* fly while following a pheromone plume. Populations overwinter in various immature stadia. The progeny of females that eclosed before 1 June can complete their life cycle in one growing season. There are morphological characteristics in the pupal stage that allow for gender identification. This information should help us develop a control strategy for *P. robiniae* in hybrid poplar.

KEYWORDS: biofix, trap color, tree height, life cycle, pupal morphology, sexually dimorphic antennae.

Introduction

Historically *Paranthrene robiniae* (Hy. Edwards) was known only as a pest in ornamental trees and nursery stock (Bentley et al. 1994). It was not until 2001 when Brown et al. (2006) reported infestations of *P. robiniae* in hybrid poplar plantations in eastern Oregon and Washington that it was considered a serious pest in an agricultural sense, thus, little of its behavior and biology is known. Other sesiid species such as the peachtree borer (*Synanthedon exitiosa* (Say)), the lesser peachtree borer (*S. pictipes* (Say)) and more recently the dogwood borer (*S. scitula* (Harris)) have been pests in orchard systems attacking peach (*Prunus*) and apple (*Malus*) respectively (Childers et al., 1979, Barry and Nielson, 1984, and Bergh and Leskey, 2003).

Paranthrene robiniae is a brightly colored diurnal moth that mimics a vespid paper wasp (Eichlin and Duckworth 1988). Larvae of *P. robiniae* burrow into the heartwood of trees, staining the wood and adding to the cost of bleaching pulp. More importantly, larval galleries weaken trees and limbs making them highly susceptible to wind damage. *Paranthrene robiniae* are especially dangerous to newly planted trees. One larva at the base of a tree can girdle the tree and weaken it, such that a small amount of wind stress can lodge the tree.

Farms grow exclusively hybrid poplar (Salicaceae: *Populus*), with the majority of the plantings consisting of clones with *Populus deltoides* (Marsh.), *P. nigra* (L.) and *P. trichocarpa* (Torr. and Gray) parentage clones. Poplar trees in this region have primarily been grown for high-quality paper pulp, but in recent yrs, with depressed price of pulp, non-structural sawtimber has become the focus for some companies (Brown et al., 2006). Depending on the end product, these poplars are grown seven yrs for pulp or twelve yrs

for non-structural sawtimber. Trees are planted during the second quarter (April to June) from “sticks,”” 23 to 33 cm cuttings of one-yr-old growth. All plantings are irrigated by drip line. Hybrid poplar in this environment grows up to 3 m in the first yr and can grow to 27 to 33 m by harvest.

Brown et al. (2006) reported a failure of chlorpyrifos to control *P. robiniae*, and proposed a pheromone-based mating disruption for control. Here we will elucidate the biology and behavior of *P. robiniae* through experimental and observation data. Questions that we addressed were: What is the annual flight period of *P. robiniae*? How many generations occur per year? How far will male *P. robiniae* fly toward a pheromone source? Do male *P. robiniae* use visual stimuli to detect mates? Where do *P. robiniae* males fly relative to the tree canopy? Are there any exploitable behaviors that will allow us to monitor and capture diurnally active female *P. robiniae*, and if so can we use these females to gain degree day information on egg development? These are all basic questions that need to be addressed before a pheromone-based control strategy can be implemented.

Materials and Methods

Terminology. Several terms will be used throughout this manuscript to describe an area of poplar trees. A plantation is the entire holding of one company, a farm is the largest contiguous subunit of a plantation, an irrigation unit will describe a managed portion of a farm irrigated by a common manifold station, a planting unit is a smaller portion of an irrigation unit, and a block will be used to describe our experimental plots.

Tree ages will be referred to by their age at the end of the growing season and not actual age of trees within said unit. So, a newly planted unit of trees will be referred to as

first yr trees because they are in their first season of growth. Trees that are in their second season will be called second yr trees, third yr trees, etc.

All experiments described used Unitrap® tri-colored (green, yellow and white) bucket traps unless otherwise specified. All traps were baited with *Scenturion* Western Poplar Clearwing Moth lures formulated by Suterra, LLC, Bend, OR. The lures are red rubber septa loaded with one mg of a 4:1 ratio of (E, Z):(Z, Z)-3,13-octadecadienyl alcohols.

Experiments were conducted from 2002 through 2006 in intensively managed hybrid poplar farms in northeastern Oregon (OR) and southeastern Washington (WA). These are high desert scrub areas that have been converted to irrigated agriculture, with water pumped from the Columbia and Snake rivers.

Color Traps. *2002.* Exploratory research was done in 2002 to determine if male *P. robiniae* use visual cues in mate location. We used two different irrigation units where trap counts had been high for several wks. In these locations, we put a “colored” bucket trap in close proximity (~1 m) to the “normal” monitoring trap. The colored bucket trap refers to a Unitrap® painted with alternating black and yellow stripes. The stripes were approximately 4 cm wide running horizontally around the trap. The normal trap refers to an unpainted, tri-color (green, yellow and white) Unitrap®. These paired traps were monitored weekly by counting and emptying trap contents. After five wks positions of traps were reversed to avoid wind direction bias. Traps were monitored for ten wks.

2003. We expanded our 2002 colored trap experiment in 2003 by adding different combinations of lures. We used six different irrigation units (65-113 hectare), in each unit we had four treatments. Treatment one “+ +” consisted of a colored trap with a lure

(+) and a normal trap with a lure (+). Treatment two “+ -” had a colored trap with a lure (+) and a normal trap without a lure (-). The third treatment “- +” had a colored trap without a lure (-) and a normal trap with a lure (+) and treatment four “- -” had a colored trap without a lure (-) and a normal trap without a lure (-). Each treatment was randomly assigned to a location that was at least 0.8 km from the nearest pheromone source. Traps were hung in adjacent trees about 2 m apart, at a height of about 2 m off the ground. Traps were monitored weekly for ten wks by counting and emptying trap contents.

We performed a similar experiment using “pane” traps (Weissling and Knight, 1994), these were normal clear acetate sheets covered with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI), and colored acetate sheets that had 20 life-size female *P. robiniae* printed on them. In each unit we had four separate treatments. Treatment one “+ +” consisted of a colored sheet with a lure (+) and a normal sheet with a lure (+). Treatment two “+ -” had a colored sheet with a lure (+) and a normal sheet without a lure (-). Treatment three “- +” had a colored sheet without a lure (-) and a normal sheet with a lure (+). Treatment four “- -” had a colored sheet without a lure (-) and a normal sheet without a lure (-). Within each unit those four treatments were randomly assigned to locations near roads that bisect the units and were at least 0.8 km from the nearest *P. robiniae* pheromone baited source. Traps hung from the branches of the trees and were collected after seven days. The *P. robiniae* males stuck to each trap were counted. The experiment was repeated three times in three different 113 ha units for a total of nine replications. At each replication new sheets with fresh Tanglefoot® were used, and either placed in a new irrigation unit, or rotated in a circular permutation if used in the same unit, in order to avoid repeated measures.

Dispersal Away From Host Plant. Bucket traps baited with 1 mg lures were placed in irrigated agricultural fields at various distances upwind from the poplar trees. The fields were planted with corn, mint, onions, or potatoes, none of which are hosts to *P. robiniae*. Traps were placed at 0, 0.2, 0.4, 0.8, and 1.21 km away from the nearest poplar trees. These traps were monitored for eight d and contents of the traps were counted and emptied five times during that period, then all traps were removed. A single trap was placed 2.01 km away from the nearest poplars. This trap was in place for nine d and contents were counted and emptied on three occasions during this period. On the ninth day this trap was removed and another trap was placed 2.82 km away from the nearest poplar trees, this trap was monitored for a period of 11 d and trap contents were counted and emptied on three occasions. We could not set traps further away from poplar trees without going on to private property or encroaching on other poplar units or riparian habitat from the opposite direction.

Height of Male Mating Flight. In the interior of units of second yr trees, bucket traps were hung from 8 m metal poles at a height of 2 m, 4 m and 6 m from the ground. We had three locations where there was a pole with three traps at the given heights. The traps were monitored weekly for 18 wks. A height measurement of the trees was also taken weekly.

Clonal Variation. Larvae of *P. robiniae* bore into trunks and limbs of poplar trees, regardless of age and clone. Pheromone baited trap catches of male *P. robiniae* were grouped by age and clone type and analyzed for differences in 2002. Tree ages were divided into two groups: first and second yr trees (≤ 2 yrs), and third yr trees and older (≥ 3 yrs). Clonal types were divided into two groups: Units that were planted with

P. deltooides x *P. nigra* (DN) parentage clones and units that were planted completely with *P. trichocarpa* x *P. deltooides* (TD) parentage clones. To analyze whether clones had differences in damage, clone types were divided into the same groups as above with the addition of units where both DN and TD clones were planted together, these will be referred to as “mixed” stands. Damage to first and second yr trees was examined for clonal differences from 2003 to 2005. Damage to trees was classified by number of *P. robiniae* entry wounds (*hits*) with frass being actively extruded.

Life History. *Phenology.* Monitoring traps baited with lures containing 1 mg *P. robiniae* sex pheromone were placed in irrigation units in March 2002-2006. Traps were placed at an interval of about one trap per 65 ha. Ninety-three traps were placed in Potlatch’s plantation (7,027 ha) and one hundred traps were placed in Boise’s plantation (6,281 ha). Starting in 2004 twenty traps were placed in Greenwood Resource’s Sandpiper farm (1,072 ha) at an interval of about one trap every 50-65 ha. All these traps were monitored weekly throughout of the flight period for five sequential years.

Monthly surveys of larval development were taken from October 2002 through February 2004. Each month we harvested one tree from a highly infested area to get a large sample of larvae. The tree was sectioned into 0.45 m logs, eight of these logs with the most visual damage were chosen for the sample. These logs were then split multiple times with an Iron & Oak® 36 ton psi log splitter (Iron & Oak Commercial Products, Inc., Streator, IL) and all larvae were extracted and returned to the laboratory alive, where weight and head capsules widths were measured. Weight was measured on a Mettler AE 100 (Mettler-Toledo, Inc., Columbus, OH) scale. Head capsule widths were measured

using a dissecting scope with an in ocular micrometer (15x). Magnification on the scope was set at 2x, where 20 units on the micrometer equaled 1 mm.

Accumulated degree-days were calculated using the single-sine method (Baskerville and Emin, 1969) and estimates of the lower threshold were made using the methodology of Jones (1991). Weather information for Potlatch's plantation, Greenwood Resources' Sandpiper farm, and Boise's Sand Lake farms were gathered from the HERO Agrimet weather station (<http://www.usbr.gov/pn/agrimet/>). The HERO Station (45° 49' 16"N 119° 31' 17"W at an elevation of 550') is located just northeast of contiguous Potlatch and Boise's Sand Lake farm and southeast of the Sandpiper farm.

Riparian Populations. Pheromone baited traps were placed in riparian habitats on the Columbia and Snake rivers to determine the flight activity of populations of *P. robiniae* in 2003, 2004, and 2005. In 2003, riparian samples were taken from four locations in Washington surrounding Boise's WA farms and from four locations in Oregon surrounding Potlatch and Boise's OR farms. In 2004 we took only riparian samples from one location in OR and one location in WA. Only one riparian location in OR was sampled in 2005. Contents from riparian traps were counted and emptied once every two wks in 2003-2004 and weekly in 2005.

Sex Determination of Pupae. Pupae of *P. robiniae* were obtained by excavating larvae from infested poplar wood. Larger larvae were placed in individual cups (Dixie® 5.5 oz (163 ml) Georgia-Pacific, Atlanta, GA) filled with pine shavings used for small animal bedding. Larvae were held in a 25°C incubator and allowed to pupate. Out of approximate 200 larvae 50 successfully pupated. These pupae were then examined under a dissecting microscope for gender differences in pupal morphology based on the

findings of Leskey and Bergh (2003). Gender was predicted for each pupa and they were placed in individual labeled cups and returned to the incubator and left to eclose. Once eclosed, adult antennal morphology was used to determine sex based on Eichlin and Duckworth (1988).

Wild female collection. On sunny afternoons, we chose a couple of fields treated with pheromone (one and two yr old trees) to search for females. The canopy in these trees reached the ground, so the whole field served as our searching area. We walked through the fields with aerial nets and captured as many *P. robiniae* (male and female) as possible.

Rearing attempt. On hot, sunny afternoons we were able to pick up female *P. robiniae* off the ground. On 7 August 2003, we collected 65 females off the ground that were aggregating around a water puddle near manifold #38 (North farm, Potlatch). These females were placed five moths to a one-gallon plastic bag, along with fresh poplar leaves, for moisture and oviposition sites. Bags were monitored daily for mortality to estimate the adult life span and presence or absence of eggs.

Salt Trap. We designed a “salt trap” based on the portable bait traps used by Austin and Riley (1995). The salt trap consisted of a fiberglass screen cylinder 1.5 m tall and 0.3 m in diameter with an enclosed screen top and an inverted screen funnel 0.3 m in diameter bottom. The cage was attached to a 0.5 x 0.5 m plywood base by nylon rope that allowed room for a 0.2 m x 0.3 m x 0.1 m aluminum pan, with another 0.1 m of clearance for moth entry. The pans were filled with sand and either tap water or a super saturated salt (NaCl) solution. Pans were refilled with water daily or as need to keep the

sand saturated with water and monitored for four weeks. Traps were hung in areas that had high populations of *P. robiniae*.

Statistical Analysis. For both color bucket trap and acetate sheet experiments, each treatment had two paired variables: one for the colored trap and one for the normal trap. In order to analyze these data we needed one number for each treatment, thus for each treatment, counts from normal traps were subtracted from the counts of colored traps. This gave us one number from each paired treatment to analyze. A positive number represents higher counts in colored traps, while a negative number represents higher counts in the normal traps. Results from the colored bucket trap experiments were analyzed with SAS using repeated measures ANOVA analysis for a Randomized Complete Block Design (PROC Glim with LSD for multiple comparisons and LSMEANs for interaction effects) (SAS 9.1). For the acetate sheet experiment, results were analyzed with SAS using ANOVA for a Randomized Complete Block Design (PROC Glim with LSD for multiple comparisons) (SAS 9.1); repeated measures were unnecessary as time was not a factor.

Height traps were monitored throughout the flight period so *P. robiniae* populations varied over time; thus data was transformed so each trap catch at a specific height represented a percentage of the total number of *P. robiniae* caught for each time period. Results from our height trap data were also analyzed using repeated measures ANOVA (PROC GLM with LSD for multiple comparisons and LSMEANs for interaction effects) (SAS 9.1). In our analyses tree height was used as a measure of time. Tree height was analyzed in increments of 1 m; during our experiment 1 m of growth was equal to 3 wk for every point until trees reached 8 m, then an additional meter in height

was equal to the last five wks of the growing season. So, percentages and standard errors presented are percentages of the total *P. robiniae* caught for the time it took the trees to grow 1m in height.

Results

Color Traps. 2002. Pheromone baited trap catches of male *P. robiniae* from colored traps nearly always surpassed that of catches from normal traps in 2002. Only during the first wk did colored traps fail to capture more *P. robiniae* males than normal traps. Rotation of traps showed that position of one trap relative to the other trap did not affect trap catches.

2003. Results from the bucket trap experiment and the acetate sheet experiment were similar. Since each treatment had two variables, a colored trap and a normal trap, in order to analyze statistically we had to use the difference in trap catch between the paired traps at each location as our single variable. Treatment effects (for example, differences between paired groups of traps, “+ +” compared to “- -”, or “+ -” compared to “- +”) for the bucket traps were significant ($F = 15.21$, $df = 3$, $P < 0.0001$) (Fig. 1). These same treatment effects for the acetate sheet experiment were also significant ($F = 5.43$, $df = 3$, $P = 0.0054$) (Fig. 2).

We compared the “+ -” and the “- +” treatments to determine the influence of pheromone on trap catch. In the bucket trap “+ -” treatment, the colored traps with the lures caught a mean of 18.82 ± 5.37 (mean \pm SE) more moths per trap per wk than traps without the pheromone lure (normal). Whereas, in the “- +” treatment, normal traps with pheromone lure caught a mean of 7.03 ± 1.84 more moths per trap per wk than colored traps without the pheromone lure. In both treatments, traps without the pheromone lure

caught a mean of 0.0167 ± 0.0168 moths per trap per wk (“+ -” treatment: normal trap without lure) and 0.0 moths per trap per wk (“- +” treatment: colored trap without lure) (Fig. 1). The same scenario happened in the acetate sheet experiment. In the “+ -” treatment, sheets with the *P. robiniae* images and a pheromone lure caught a mean of 20.22 ± 10.52 more moths per sheet than sheets without the images or pheromone lure. In the “- +” treatment, blank sheets with pheromone lures caught a mean of 20.55 ± 8.45 more moths per sheet than sheets with images but without the pheromone lure. Normal sheets without the pheromone lures caught a mean of 1.88 ± 1.09 moths per sheet in the “+ -” treatment (normal sheets without lure) and a mean of 2.89 ± 2.09 moths per sheet in the “- +” treatment (colored sheets without lure) (Fig. 2). Thus traps baited with a lure caught more moths than traps without a lure regardless of color.

We compared the “+ +” and “- -” treatments to further examine the effect that color had on trap catch. For the “+ +” treatment, where both bucket traps had pheromone lures the colored traps caught a mean of 7.25 ± 3.75 more moths per trap per wk than the normal traps. In the “- -” treatment, where neither bucket trap had a pheromone lure, there was a mean of 0.0167 ± 0.0168 more moths per trap per wk caught in colored traps (Fig. 1). Colored acetate sheets in the “+ +” treatment caught a mean of 3.67 ± 2.44 more moths per sheet than normal sheets and in the “- -” treatment, where neither sheet had lures, colored sheets again caught a mean of 0.11 ± 0.12 more moths per sheet than normal sheets (Fig. 2).

Trap catch varied significantly ($F = 5.64$, $df = 5$ $P < 0.0001$) from unit to unit and there were significant ($F = 5.92$, $df = 15$ $P < 0.0001$) treatment by unit interaction effects in regards to trap catch in the colored bucket trap experiment, but both of these results are

expected with population sizes varying from unit to unit. There were no significant time effects or time by treatment effects ($F = 0.91$, $df = 9$, $P = 0.5168$; $F = 1.14$, $df = 27$, $P = 0.3020$, respectively) in the colored bucket trap experiment. Trap catch did not vary significantly ($F = 0.24$, $df = 8$, $P = 0.9801$) from unit to unit in the acetate sheet experiment.

Dispersal Away From Host Plant. Multiple *P. robiniae* males were caught at each trap location. A total of 219 male *P. robiniae* were caught at the edge of poplar plantings (0.0 km). Trap catches decreased as the distances away from poplar trees increased. However, multiple *P. robiniae* males were caught at each distance away from the nearest poplar trees out to a distance of 2.82 km.

Height of Male Mating Flight. Initially, most ($90.78 \pm 7.96\%$) male *P. robiniae* moths were caught in pheromone baited traps attached 2 m above ground to an 8 m metal post. As the canopy height increased, the percentage of *P. robiniae* males caught in the traps set at 2 m above ground decreased while the percentage caught in traps placed at 4 m increased (Fig. 3). When canopy height reached 7 m, the percentage of *P. robiniae* caught ($60.74 \pm 2.18\%$) in traps set at 4 m surpassed the percentage of moths captured ($21.48 \pm 1.60\%$) in the 2 m traps. Traps attached to the metal posts at 6 m consistently caught fewer *P. robiniae* males until tree canopy height reached 7 m. Although, the percent caught at each height were not significantly different when tree canopy exceeded 8 m, the 6 m traps caught $42.85 \pm 3.49\%$ of *P. robiniae* while the 4 m traps caught $40.0 \pm 4.77\%$ (Fig. 3). We found that there was a significant ($F = 17.90$, $df = 8$, $P < 0.0001$) change in where (trap height) the highest percent of male *P. robiniae* were caught over time (tree height).

Clonal Variation. Overall, we found a significant interaction effect between age and clone type ($F = 5.66$ $df = 1$ $P = 0.0198$) for season long trap catches in 2002. In third yr trees and older there was a significant difference ($P < 0.001$, from LSMEANs) in mean trap catch between DN (241.81 ± 39.16 *P. robiniae* per trap) and TD (863.6 ± 84.97 *P. robiniae* per trap). Thus, in older plantings, there were more *P. robiniae* captured in traps positioned in units planted with indigenous *P. trichocarpa* parentage. In first and second yr trees, mean trap catch did not differ whether the clone was DN (917.8 ± 164.85 *P. robiniae* per trap) or TD (967.8 ± 177.32 *P. robiniae* per trap). Although not significant ($F = 0.79$ $df = 1$ $P = 0.46$), we found more damage in DN clones (0.54 ± 0.12 hits per tree) than TD (0.22 ± 0.07 hits per tree) or in mixed clone units (0.42 ± 0.22 hits per tree) in first and second yr trees.

Life History. Our phenology model for eastern Oregon and Washington predicts the first male *P. robiniae* will be detected in pheromone traps between 114 and 145 degree-days. Over the past five yrs the first moth was captured either the second or third wk of April (Table 1). The flight period continues throughout July without a discernable peak but with weekly fluctuations in mean trap catch occurring on large farms (>1,000 ha). On smaller farms (<1,000 ha) we saw a more distinct peak in the *P. robiniae* male flight period (Fig. 4); although, the entire length of the flight period is the same. In late August the flight period begins to taper off, but male *P. robiniae* continue to fly well into October (Table 2).

In riparian areas the populations of *P. robiniae* appears to be much smaller than on the hybrid poplar farms; at the least, fewer moths are caught per pheromone baited traps. During three yrs of trapping in riparian areas, we caught a mean of 1.4 ± 0.16 male

P. robiniae per trap per wk. The male flight period in these riparian areas starts early in May and goes on through August, similar to what has been recorded for poplar plantations.

Frequencies of head capsule widths from our survey show no distinct divisions between instars. The destructive sampling methods we used in our survey, favored finding larger more apparent larvae. Head capsule widths range from 0.50 mm to 3.5 mm which probably only represents instars three through five (Fig. 5). There is also a large variation in overwintering larval head capsule size, suggesting *P. robiniae* overwinter in variable stages of development, causing irregular first emergence patterns (Fig. 6).

Sex Determination of Pupae. Fifty pupae were examined; the predicted gender of each was recorded as either male or female. Each pupa was held in a labeled cup and placed back into the incubator. Only sixteen adult *P. robiniae* eclosed; ten females and six males. The gender of all sixteen adults was correctly identified by pupal morphology. Female *P. robiniae* have a double row of spines on abdominal segments 2-6 and a single row of spines on fused segments 7-10. Male *P. robiniae* have a double row of spines on their abdominal segments 2-7 and a single row of spines on fused segments 8-10. Thus the key to gender is whether the 7th segment has one (female) or two (males) rows of spines.

Wild Female Collection. Four adult *P. robiniae* were caught by surveying fields, and only two of those were female. These numbers did not provide us with enough adult *P. robiniae* numbers to warrant continuing with this monitoring technique.

Rearing attempt. Of the 65 field captured female *P. robiniae* moths, most died within 24 hrs and all died after the 3 d, and no eggs were laid.

Salt Trap. Over the course of the four wks we monitored our salt trap, we only caught one female *P. robiniae*. Various other moths and flies were captured, but unidentified.

Discussion

Visual Cues. *Paranthrene robiniae* males use female sex pheromone for mate location. Similar to other sesiid species, the male *P. robiniae* is very sensitive to the females' sex pheromone. However, since *P. robiniae* are brightly colored and diurnal, there is a possibility of visual mate detection, and other sesiid species have been caught in traps colored similar to their mate's coloration (Childers et al. 1979). Other diurnal Lepidoptera such as butterflies often rely on visual cues for mate detection (Jiggins et al. 2001, Fordyce et al. 2002,). Rutowski et al. (2001) showed that males of *Asterocampa leilia* (Lepidoptera: Nymphalidae) could visually detect mates at a distance of up to 3 m.

High populations of pests are always a concern when implementing a pheromone control strategy because, there is the possibility of mating occurring when males and females eclose in close proximity to one another. We have observed over thirty pupal exuviae within a 0.3 m linear section of a tree (Fig. 7). This density of moths could allow for chance encounters of males and females eclosing together in close enough proximity where visual stimulation invites courtship behavior. A high pest density of a brightly colored diurnal species may allow for increased visual mate detection overriding the effectiveness of a pheromone control strategy.

Our experiments on *P. robiniae* visual detection of a mate suggest that although color (black and yellow) may increase trap catch slightly, chemotaxis is a more important cue at least at distances greater than two meters. Knight and Miliczky (2003) and

Suckling et al. (2005) found certain colors may effect trap catch and that traps without pheromone were ineffective in catching crepuscular or nocturnal moths. However, neither of these studies examined how trap color might influence moth capture in nearby traps. In other words, could a “normal” trap loaded with the sex pheromone placed near a “colored” trap without a lure, result in pheromone attracted moths being captured in the colored trap that does not have a pheromone source?

The “+ -” and the “- +” treatments of both the bucket trap and acetate sheet experiments support the need for the sex pheromone to capture male *P. robiniae*. In both treatments the trap that had the pheromone lure (+) caught more *P. robiniae* males than the trap without the pheromone lure (-), regardless of trap color. In fact, in the bucket trap “+ -” treatment, the normal traps without the pheromone lure (-) only caught one moth total, and in the “- +” treatment the colored traps without the lure (-) caught no moths for the entire period the experiment was run. Acetate sheets without lures (-) in the “+ -” and the “- +” treatments caught a mean of 1.89 ± 1.09 and 2.89 ± 2.09 moths per trap, respectively. Thus a colored bucket trap without a lure positioned near a normal pheromone baited trap failed to attract male moths, but acetate sheets with images of female *P. robiniae* only, did capture some males moths when positioned near normal acetate sheets baited with pheromone.

These acetate sheets may have been more effective in capturing moths. Brown et al. (2006) reported that Delta traps were more effective in capturing male *P. robiniae* than bucket traps. The acetate sheet traps that we used had a similar trapping mechanism to Delta traps. The sheets used Tanglefoot®, so if the moth just touches the sheet it will be caught, whereas the bucket traps rely on the moths actually entering the trap. So it is

possible that with more male *P. robiniae* in the vicinity, unbaited acetate sheets with printed images of mates are more likely to capture male moths than unbaited colored bucket trap simply because of the difference in trapping mechanisms. However, the number of moths captured on the acetate sheets without the pheromone lure regardless of color, were negligible compared to moths captured on acetate sheets with pheromone lures (Fig. 2).

Moth coloration could become a problem for pest management if the moth can use its mate's coloration to bypass pheromone based control strategies. The basis of a pheromone control strategy is to mask the presence of females from males by inhibiting the males' mode of mate detection. If a male has two modes of mate detection, olfactory and visual, and the control strategy only inhibits one mode, then a pheromone based strategy may fail. Our colored trap experiments suggest that although *P. robiniae* may use visual cues in mate recognition, this fact will not inhibit the effectiveness of a pheromone control strategy. Barry and Neilson (1984) showed that visual orientation of the male peachtree borer (*Synanthedon exitiosa*, Say) to the female was not used until within 10 cm of the mate. The results of our experiments suggest that color recognition is not effective at the distance of two m or greater in *P. robiniae*. Our results also suggest that a pheromone control strategy should not be affected by the fact that the *P. robiniae* may use visual cues in mate location at close range.

Dispersal Away From Host Plant. We have shown that male *P. robiniae* will fly at least 2.82 km away from their host plant toward a conspecific sex pheromone source. It was not possible to place traps further away from the poplar farms without encroaching on other parts of the farm or riparian areas from other directions. We believe male *P.*

robiniae would fly even greater distances to locate a mate, and from this observation, we can make a couple of inferences about the *P. robiniae* male behavior. The sex pheromone of *P. robiniae* was synthesized specifically for this moth (Cowles et al. 1996), and male *P. robiniae* are very sensitive to the synthetic female sex pheromone. If the pheromone is not species specific, troubles in pest monitoring may occur due to incorrect pheromone blends (Bergh et al. 2004, Zhang et al. 2006). Anecdotal evidence of *P. robiniae* male sensitivity to the pheromone include repeated observations that when traps are placed into an area where no traps were previously, trap catches are very high the first couple of days and then levels fall off with fewer moths captured on subsequent days. Another inference is that because of the distance males will travel, immigration and infestation of a new area could happen relatively fast. *Paranthrene robiniae* was virtually unknown as a pest, except in ornamentals, until 2002 (Bentley et al. 1994, Chastagner and Hudaki 1999, Coyle et al. 2005, Brown et al. 2006). Its mobility is one of the factors that allowed the *P. robiniae* to immigrate and infest the poplar plantations from its native riparian habitat in such a short time. Infestations of new habitat is probably limited by the ability of the females to disperse, once relocated, a male moths will respond females calling.

Currently, we have no way to attract diurnally active female moths to any sort of trap so “distance trapping” was not possible. We were able to make visual observations of female mobility, when disturbed these females were very mobile and just as quick and agile of fliers as male *P. robiniae*.

Height of Male Mating Flight: The results from our height study suggest that male *P. robiniae* fly at or just below canopy level when following a pheromone plume.

These data have shown that as tree height increased during the flight period the trap height that caught the majority of *P. robiniae* also increased (Fig. 3). We have also observed that male *P. robiniae* fly from late morning until early evening, with both observations suggesting their flight activity is related to light intensity. A laboratory observational study required a 400-watt high-pressure sodium grow lamp (Sylvania®, Danvers, MA) placed about 1 m above the cage to initiate mating behavior. Under this intense light, females would start calling four hrs into the light cycle at which time the males would also become active. Flying at or near canopy level would therefore give *P. robiniae* the most exposure to the sun's radiation. Anecdotally, when we were able to catch female *P. robiniae*, we found them “basking” on sunlit parts of trees on the road edges.

Clonal Variation. Regardless of *P. robiniae*'s mobility, our trap capture and survey results are from large (65 to 110 ha) units of uniform age and clones of hybrid poplar. Thus, based on trap capture and damage surveys we believe that poplar clones with *P. trichocarpa* parentage are more suitable for *P. robiniae* infestations than clones with DN parentage. DN clones did sustain twice as much damage at an early age; however, pheromone baited traps within TD clones in older trees captured almost four times more male *P. robiniae* compared to traps placed in older DN clones. We found no evidence of differences in yearly trap catch of *P. robiniae* in first and second yr trees regarding clones, although not significant, there was actually more damage found in DN units with younger trees. In older trees (≥ 3 yrs) we did find a difference in yearly trap catch, traps in DN units caught about one third less *P. robiniae* than units of TD clones. Although no damage surveys were carried out in units with older trees because of the

trees' size (10 – 15 m), observationally, DN units have noticeably less damage than trees with TD parentage. These data suggest there are some age related mechanisms that develop in the DN clone that provide an older tree a level of resistance to *P. robiniae* that they do not have as first or second yr trees. Poplar clones with the parentage of *P. trichocarpa* have been shown to be more susceptible to the weevil, *Cryptorhynchus lapathi* (Linnaeus) (Johnson and Johnson, 2003, Broberg and Borden 2005, Broberg et al. 2005), which is also a pest in our experimental area. *Cryptorhynchus lapathi* weevils have similar host preferences as *P. robiniae*.

Life History. Our *P. robiniae* phenology model is based purely on field data, which may limit its precision. The traps were monitored weekly, which allows seven days of error, and during the growing season 5-13 heat units can accumulate each day. When combined, weekly monitoring and rapid increases of degree days (DD) could translate to a 35 to 91 DD difference in one wk, the time between trap monitoring. It is possible that after control measures reduce *P. robiniae* population sizes, the first emergence patterns will become more consistent and the mating season will be more restricted, such as seen on smaller farms (Fig. 4). Observational data suggests that *P. robiniae* has one generation, with the possibility of a partial second generation each yr (Table 2). Female *P. robiniae* are active early in the season (late April to mid-May) and their progeny may be responsible for the population trapped at the end of the season. We have found pupae at the end of August in first yr trees. Pupae in first yr trees suggest that *P. robiniae* can mature from egg to adult in one season. We have also found *P. robiniae* contamination in planting sticks. These larvae have all been late instar individuals

suggesting they overwintered within the planting sticks, and may contribute to an early infestation (May) of newly planted units.

Head capsule size in *P. robiniae* seems to be very plastic. Results from our monthly survey show no clear-cut divisions in the frequency of head capsule width (Fig. 5 and 6). Laboratory rearing needs to be done in order to further discriminate divisions in head capsule width.

Sex Determination. Sex determination of *P. robiniae* in the field can be difficult given they are not distinctly sexually dimorphic, unlike some other sessid species. For example peachtree borer females are black with a single orange strip on their abdomen, while males are a more general wasp mimic (Russell and Stanley 1969, Eichlin and Duckworth 1988). *Paranthrene robiniae* are sexually dimorphic, however the differences are slight. As with other sessids there are antennal differences between males and females; both males and females have clavate antennae, however, males' antennae are ciliate on the ventral side (Eichlin and Duckworth, 1988). Another difference is the anal scale tuft, which the male has and can either hold together or spread out fan-like (Eichlin & Duckworth 1988). In the female *P. robiniae*, the anal scale tuft is reduced. If the male *P. robiniae* does not have the anal tuft spread open, the posterior abdomen of the male and female looks similar.

Identifying sex in immature stages is even more difficult. There are no apparent external morphological differences in larvae. Carpenterworm moth, (*Prionoxystus robiniae* (Peck)) another pest species of poplar, has a size dimorphism in mature larvae that is also present in the adult (Leppla et al. 1979). Other lepidopteran larvae, such as the codling moth, have pigmented testes visible through the integument of the males

(Reed-Larson and Brown 1990). We observed differences in pupal morphology similar to those in dogwood borer (*Synanthedon scitula* [Harris]) reported by Leskey and Bergh (2003). The pupal differences we have found make identifying sex in *P. robiniae* in an immature stage easier. In the field identification of pupae or pupal cases can be made visually or with the aid of a hand lens. This will also make distinguishing sexes for future laboratory behavioral studies easier.

Wild Female Collection. *Rearing attempt.* We assumed that the females captured from the aggregation at manifold #38 would lay eggs, and then we could monitor the length of time from oviposition until eclosion of larvae, while monitoring daily minimum and maximum temperatures so that we could develop a degree day model for egg development. This model would have allowed us to correlate damage we observed in the field to a developmental model, since damage is only the result of larval feeding

After several attempts, we hypothesized that all the female *P. robiniae* that we were capturing from these aggregations were probably “old.” Old, meaning that they had already mated and laid all their eggs. These suspicions were reinforced by Lesky et al.’s (2003) results, which reported that the dogwood borer females mate and deposit eggs on the same day they emerge. So if the same were true for the female *P. robiniae*, we would not have seen these females aggregating until after their eggs had already been laid.

Adult *P. robiniae* have a short lifespan. In our attempts to keep adults alive, most of the *P. robiniae* we captured died within 24 hrs and none survived more than five days. We believe that this was partly due to their age before we captured them while “puddling” behavior. The *P. robiniae* we were able to rear out from larvae collected from

logs, only survived two or three days with a few surviving up to five days. Future attempts toward rearing *P. robiniae* could benefit from Pritchard and Meyer (2003) and Leskey et al. (2003) with their experiences rearing grape root borer and dogwood borer, respectively.

Salt traps. We have observed *P. robiniae* aggregated around water sources. Upon closer inspection we realized that all the *P. robiniae* aggregating around a water source were females. This led to our effort to use a “salt trap” to capture female *P. robiniae*. Puddling is a well-documented behavior in Lepidoptera (Smedley and Eisner, 1995). It occurs in many species of butterflies and moths. It is better documented in butterflies because they are diurnal, making observations easier than with the nocturnal moths. Puddling is a behavior where the male visits standing water, urine, dung, etc. to take up sodium and amino acids, which they then pass on to the female via the spermatophore (Smedley and Eisner, 1995). The natural selection reward for this behavior is linked to providing essential nutrients to the female and ultimately to their progeny. This behavior has been documented with females in a few species but is extremely rare (Scriber, 2002).

Puddling is believed to serve as a way to replenish “vigor” after mating and egg laying in old females (Scriber, 2002). We believe this to be the case with *P. robiniae* also. Female *P. robiniae* are very active moths, which probably require higher sodium levels than normal. We had planned to exploit this behavior by constructing traps used to catch female *P. robiniae* with sodium (NaCl) as bait, however our pheromone control strategy reduced the population to the point where it could not be tested any further. Our salt traps yielded only one female *P. robiniae*, and at the time of our salt trap deployment, pheromone baited monitoring traps for male *P. robiniae* shut down, suggesting a general

population decline and thus our attempts to capture female *P. robiniae* may have been poorly timed. Further attempts to capture female moths with salt traps were halted due to declining populations of *P. robiniae* in subsequent years.

Another possibility for the puddling behavior may be attributed to the arid conditions where these hybrid poplars are grown. The hybrid poplars in this region are grown in a high desert scrub climate, which experiences little annual precipitation. The poplar trees are provided with water through drip irrigation. The puddling behavior observed in female *P. robiniae* in the hybrid poplar plantations may be exaggerated because of the lack of water. In riparian areas, the natural habitat of *P. robiniae*, puddling areas are presumably more readily available. Whereas, in the hybrid poplar system puddling areas are fewer and farther between, possibly causing female *P. robiniae* to aggregate around an available water source.

Conclusions: In summary, we have found *P. robiniae* males may use visual mate detection, however only at close range, and this fact should not affect a pheromone control strategy. *Paranthrene robiniae* males are very receptive to the female sex pheromone and males fly at or just below canopy level. There is evidence that suggesting that certain hybrid poplar clones are more suitable for *P. robiniae*. We have also found immature and adult characteristics that will help in identifying sexes of *P. robiniae*. Female *P. robiniae* are difficult to monitor and with the reduced population due to the pheromone control strategy, this problem may not be resolved. From field data and observations, we have put together a phenology of *P. robiniae*. All of which should help fill in the gap in the available literature, and should help when devising successful control strategies for *P. robiniae*.

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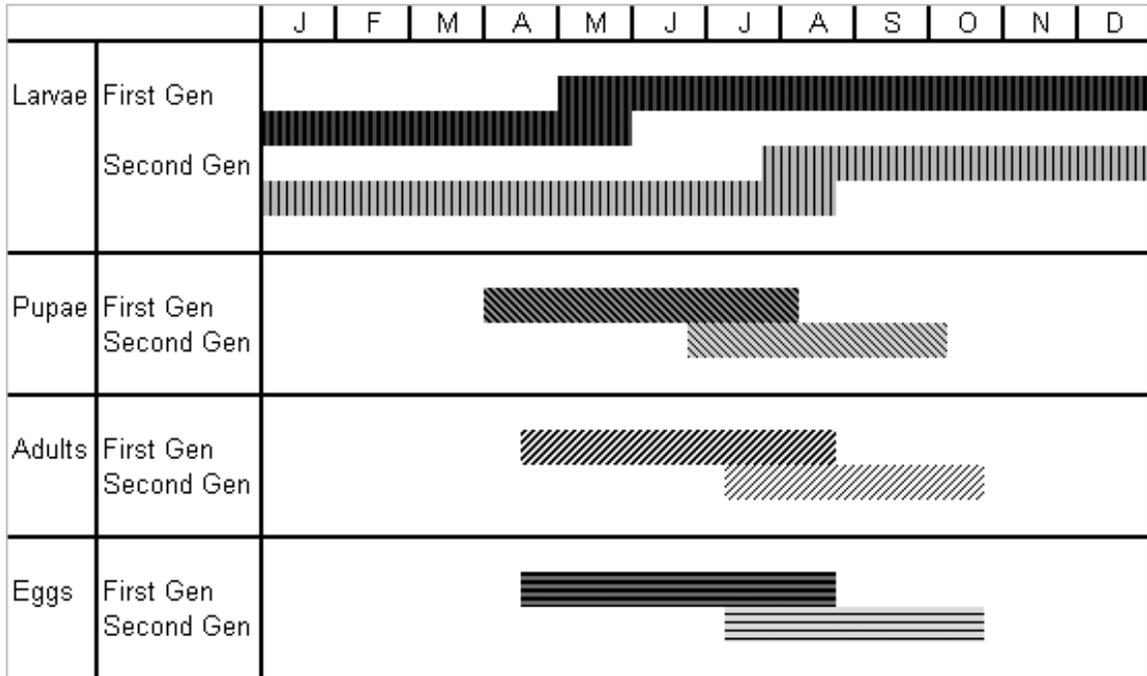
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Tables:

Table 1: Five yrs of first capture data for male *P. robiniae*. Including the farm where first capture was made and date. All degree-day calculations were made from 1 January using the ‘single-sine’ method with 10°C minimum threshold (Oregon State University 2004). All temperature information was gathered from the “HERO” Agimet weather station (45° 49’ 119” N; 119° 31’ 17” W). Degree-days minus seven days is also given as the time in between dates of trap monitoring.

Year	Farm	Date of First Capture	Degree-Days	Degree-Days -7 days
2002	Sand Lake (Boise)	24 April	121.4	99.8
2003	Potlatch	12 April	117.3	91.6
2004	Sandpiper (GWR)	17 April	149.6	125.6
2005	Sand Lake (Boise), Potlatch	12 April	110.2	91.5
2006	Sand Lake (GWR)	12 April	114.3	89.6

Table 2: Diagram representing the life-cycle of *P. robiniae*. The length of time the adults are present is based upon four yrs of 1 mg pheromone baited trap capture. Egg and pupal duration is based upon the adult flight period. Larval data are based upon an 18 month destructive sampling survey. The second generation is proposed from our field observations and observational laboratory rearing study.



Figures:

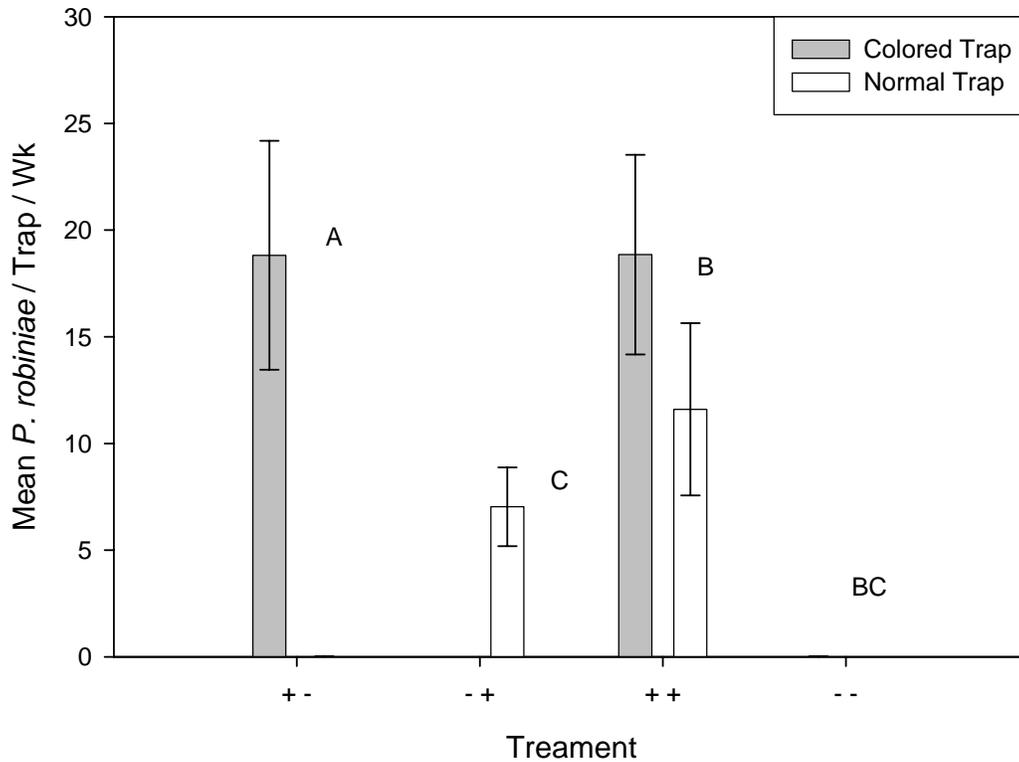


Figure 1: Results of the colored bucket trap experiment. Shaded bars always represent “colored” traps and white bars always represent “normal” traps. The first symbol in the paired treatment represents presence (+) or absence (-) of a pheromone lure in the colored trap and the second symbol represent the represents presence (+) or absence (-) of a pheromone lure in the normal trap. Analyses are done on the difference between the paired traps for every treatment. Means with the same letters are not significantly different at $P < 0.05$.

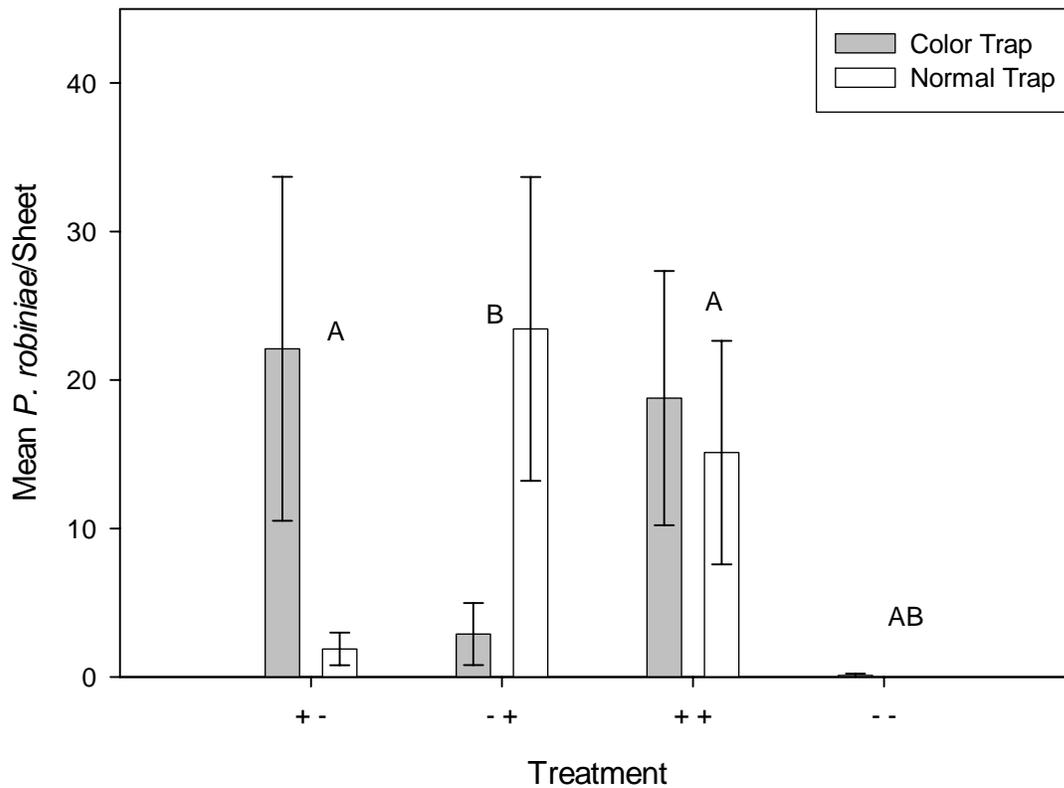


Figure 2: Representation of the colored acetate sheet experiment. Shaded bars always represent “colored” sheets and white bars always represent “normal” sheets. The first symbol in the paired treatment represents presence (+) or absence (-) of a pheromone lure in the colored sheet and the second symbol represents the presence (+) or absence (-) of a pheromone lure in the normal sheet. Analyses are done on the difference between the paired traps for each treatment. Means with the same letters are not significantly different at $P < 0.05$.

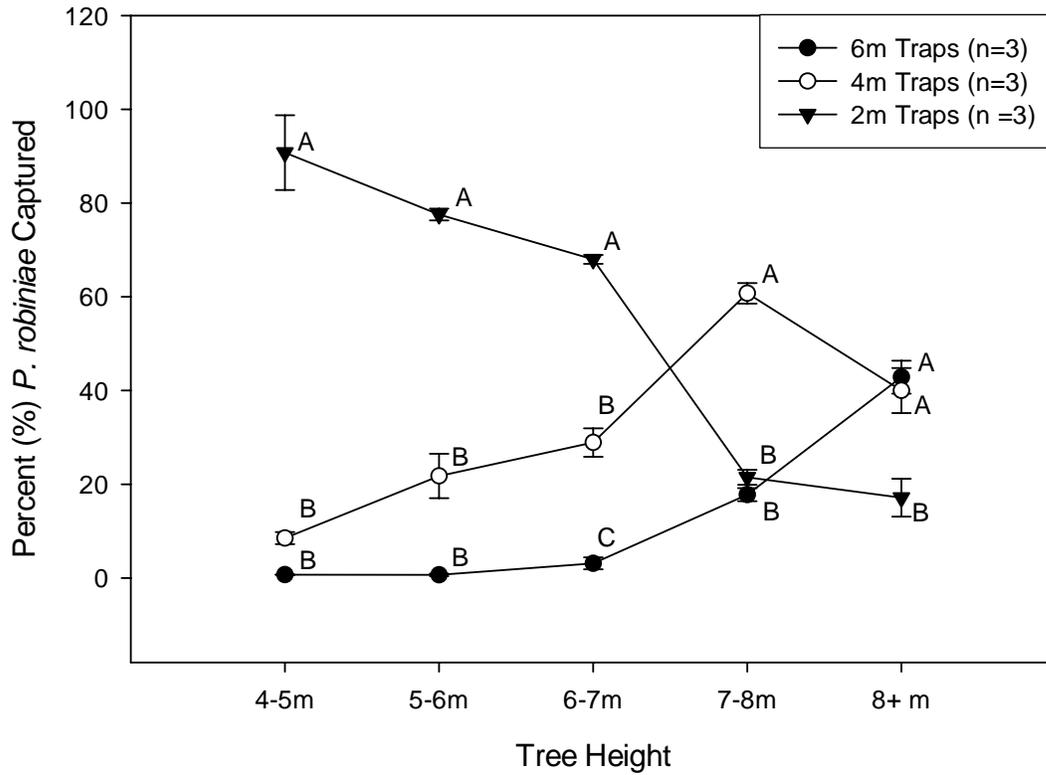


Figure 3: Male *P. robiniae* mating flight height changed during tree growth throughout the season. Each meter in tree height represents three sequential wks except the 8+m represent the last five wks of the season. Means with same letter at the same tree height are not significantly different at $P < 0.05$.

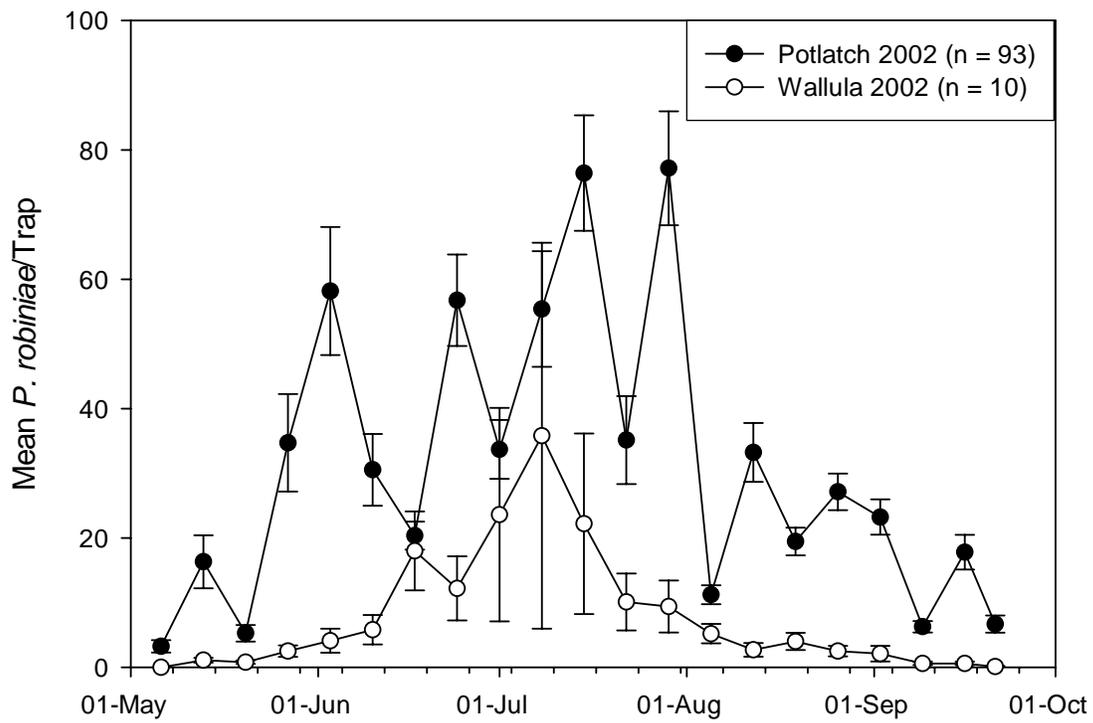


Figure 4: Differences in seasonal trap catch of male *P. robiniae* on a large farm (Potlatch 6,728.5 ha) compared to a small farm (Wallula 466.48 ha). Note the single peak with a shoulder at Wallula compared to the multiple peaks at Potlatch.

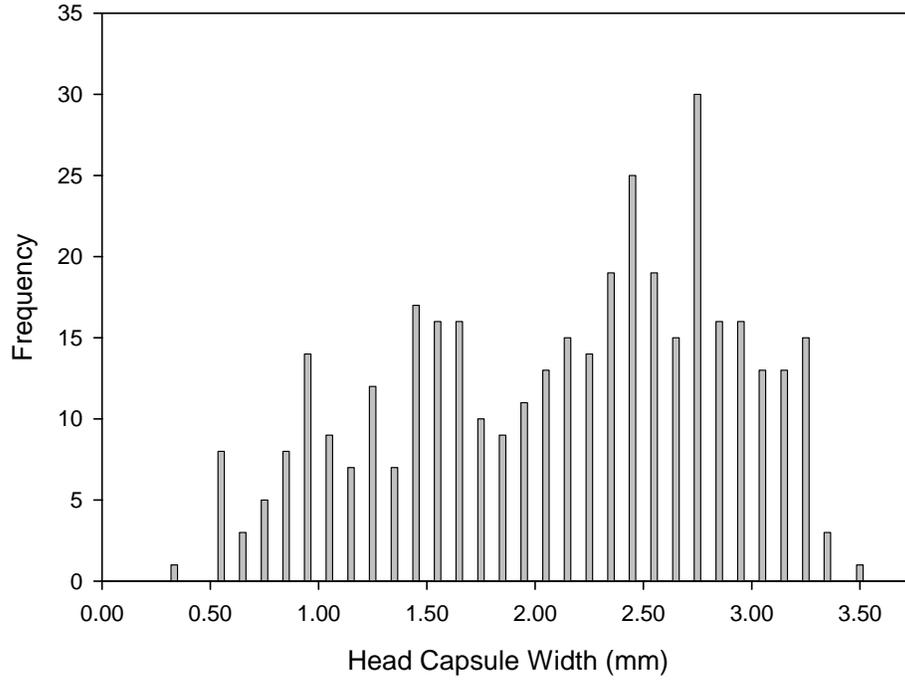


Figure 5: Frequency diagram of *P. robiniae* larval head capsule widths collected using monthly (October 2002 through February 2004) destructive sampling.



Figure 6: Variation in *P. robiniae* overwintering stage. These specimens were extracted from a tree cut in January 2003. Larval representation is from about third to fifth instar. The pupa was viable at the time of this photo, but did not eclose to an adult.



Figure 7: Representation of a poplar tree heavily infested with *P. robiniae*. There are 30 *P. robiniae* pupal cases extruding for the 1.5 m section of trunk.

**Chapter 3: An endemic population of western poplar clearwing moths
(Lepidoptera: Sesiidae) invades a monoculture of hybrid poplar.**

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ABSTRACT: Western poplar clearwing moths [*Paranthrene robiniae* (Hy. Edwards)] endemic in Pacific Northwest riparian habitats at low population densities, rapidly colonized commercial hybrid poplar plantings. Populations increased quickly, and this moth became a major pest. Trap catches of male moths in mid-season surveys increased 190-fold from 95 in 2001 to over 18,500 in 2002 across 6,597 hectares (ha) of poplars monitored. The outbreak of western poplar clearwing moth was widespread in 2002. Pheromone baited traps placed 1 trap per 81.75 ha over 13,274 ha of commercial poplars, captured >108,000 male moths in 2002. Damage to commercial poplars included girdling of saplings and burrows in limbs and trunks of trees. Repeated applications of chlorpyrifos failed to reduce the abundance of moths in 2002. Two management strategies over two separate plantations of approximately 6,500 ha each were contrasted. Future control strategies recommend a halt to the use of contact insecticides that target adult moths. Short-term (3-5 years) control should involve a pheromone-based mating disruption strategy followed, eventually, by selection of a clone that is less susceptible to *P. robiniae* attack.

KEY WORDS: integrated pest management, outbreak, chlorpyrifos, mating disruption

Introduction

Poplars are grown for use in paper, reconstituted wood panels, plywood, pallets, furniture, fuel, and non-structural sawtimber. Over 14,000 ha of hybrid poplar are currently under cultivation in the Pacific Northwest of the United States, contributing to the economy of northeast Oregon near Boardman and southeast Washington near the cities of Paterson, Wallula, and Burbank. Trees grown at these lower elevations are accessible year-round; water is readily available from the Snake and Columbia river systems above and below their confluence; the leeward side of the Cascade Mountains averages >300 cloudless days/year; and rotation for pulp can be as short as 7 years. Producers cannot tolerate insect feeding that endangers the establishment of saplings, weakens limbs, or defoliates trees to an extent that maximum growth is not realized.

Potlatch Corporation, a major producer of poplar, has converted its operations near Boardman, Oregon from growing trees for pulp toward harvesting sawtimber. This strategy lengthens crop rotation from 7 to 12 years and adds substantial costs to the production cycle by adding cultural practices, including the pruning of each tree four times to a height of 8 m. Poplar has desirable milling properties that will allow larger logs to be veneered. A shift in pest management practices (Stanton et al. 2002) is now required because insect pests that excavate galleries into the heartwood, vector diseases that discolor wood, or damage dominant terminal shoots can no longer be tolerated as they were when production was solely for pulp.

Insects have always posed a major threat to hybrid poplar monocultures, but concern has multiplied as harvest rotation is lengthened by 5 years and clear heartwood, rather than chips, is the desired end product. Retail stores (Washburn and Miller 2003)

that will market home products made from poplar are requiring trees be managed under the guidelines of the Forest Stewardship Council (FSC). The FSC prohibits the use of World Health Organization Type 1A, 1B, and chlorinated hydrocarbon pesticides. FSC restrictions on pesticides are dynamic. FSC has restricted the use of many broad-spectrum pesticides, but the organophosphate chlorpyrifos is permitted.

Research toward developing a poplar integrated pest management (IPM) program was initiated in 2001. Initial surveys were conducted to determine arthropods of economic significance. Data were collected weekly from April through September using light traps, pit-fall traps, pheromone-baited traps, and visual inspection of trees. We determined that the principal pests were three heartwood-burrowing insects: two Lepidoptera (western poplar clearwing moth, Sesiidae, *Paranthrene robiniae* (Hy. Edwards) and carpenterworm moth, Cossidae, *Prionoxystus robiniae* (Peck)) and one Coleopteran species (poplar/willow borer, Curculionidae, *Cryptorhynchus lapathi* (L.)). We decided to direct our initial control efforts toward the clearwing moth, because its population was widespread in all plantations surveyed.

There are numerous Sesiidae species that damage agricultural crops and shrubs, including the peach tree borer, *Synanthedon pictipes* (Grote and Robinson); the dogwood borer, *S. scitula* (Harris); currant borer, *S. tipuliformis* (Clerck); and grape root borer, *Vitacea polistiformis* (Harris) (Anderson et al. 1987, Barry and Nielsen 1984, Cossentine et al. 1990, Potter and Timmons 1983, Reed et al. 1988). Clearwing moths are generally not considered pests in their natural habitat, but when potential hosts are planted within their range, they can become a pest. For example, there is a low incidence of dogwood borer damage to native dogwood (*Cornus florida*) in the understory of eastern deciduous

forest (Rogers and Grant 1990), but *S. scitula* has become a serious pest of apples in Appalachian states (Bergh and Leskey 2003). Johnson et al. (1991) suspected feral female grape root borers (*V. polistiformis*) originating from wild grapevines were invading bunch grape vineyards in Arkansas. Eastern cottonwood *Populus deltoides* (Bartr. ex Marsh.) nursery stock can be heavily infested with the cottonwood clearwing borer *Paranthrene dollii* (Neumoegen), but damage in plantations is of less concern (Coyle et al. 2005).

Multiple attacks can severely weaken stems, limbs, and bole of a tree so that windstorms can blow down part or all of an infested tree. One clearwing moth gallery can downgrade a veneer-quality saw log to chipping status for pulp. In addition to this obvious damage in older trees, clearwing larvae can girdle newly planted trees. Once the unit has been prepared for planting, laborers insert “sticks” at each drip emitter by hand. A small-diameter sapling does not allow burrowing directly into the heartwood, instead the clearwing moth larva creates a circumferential burrow at cambium depth that girdles the stem. These trees are very evident because they are prone to being lodged by moderate gusts of wind. Even if there were enough cambium tissue to sustain growth, the lodged saplings will be sprayed with herbicides used to reduce weed competition between rows. Replanting requires the same amount of labor-hours to survey the entire unit for girdled plants, remove the damaged rootstock, and insert a fresh cutting as initial planting. Some irrigation units suffer >20% loss to this girdling activity and these trees cannot be replaced once the canopy closes in late June, thus that space remains vacant for 7 to 12 years.

Our research was designed to determine the extent of the clearwing population in hybrid poplars and to examine if a contact insecticide would control the pest. Here we: 1) report a classic example of an endemic species invading a monoculture to become a major pest problem; 2) document the ineffectiveness of broad-spectrum insecticides to resolve the situation; and 3) make recommendations for new strategies toward controlling western poplar clearwing moth populations.

Material and Methods

Terminology. Throughout this manuscript we have used several terms to describe an area of poplar trees. We used the term “plantation” to describe the entire holdings of one company, *e.g.*, Boise’s or Potlatch’s plantation. The term “farm” refers to the largest subunit of a plantation, *e.g.*, Boise’s Wallula farm or Potlatch’s north (2,700 ha) and south (3,900 ha) farms. We used “unit” to denote a management parcel of land irrigated by a common pump and planted at the same time; these are usually 65 to 113 hectares in size.

Physical description of plantations. Boise Corporation had 7,353 hectares of poplars in production in 2001. There were six separate farms, two (Sand Lake and Finley Butte) in eastern Oregon and four (Sand Piper, Wallula, Hill Top, and Ice Harbor) in eastern Washington. Sand Piper (1,072 ha) and Wallula (467 ha) were isolated farms. Hill Top (1,648 ha) and Ice Harbor (1,832 ha) were contiguous farms in Washington. Sand Lake (1,400 ha) and Finley Butte (935 ha) farms were contiguous with one another as well as with Potlatch’s plantation (6,620 ha), together representing ~9,000 contiguous hectares of poplar production near Boardman, Oregon. Potlatch’s irrigation units are squares of either 65 or 113 hectares each with roads that transect the center connecting

pumping stations and run between unit borders, so that each unit has four distinct quadrants of either 16 or 28 hectares. Boise's pumping stations are often located between adjacent, irregularly shaped irrigation units of various sizes from 14 to 373 hectares each. All plantations are on sandy soils, originally high desert containing sage (*Artemisia* sp.), rabbit brush (*Chrysothamnus* sp.), and related vegetation. Trees are provided with fertilizer and drip irrigated 10 to 24 h/day depending upon age and time of year. Hybrid poplar clones are of *Populus trichocarpa* Torr. and Gray, *P. deltoides*, and *P. nigra* L. parentage, all are in the family Salicaceae. Trees planted in April can exceed 5 m by October and at harvest can reach 35 m in height.

Pheromone baited traps. Ninety-two pheromone-baited "delta traps" (Scenturion, Clinton WA) were deployed, one per irrigation unit, in Potlatch's plantation in 2001. We baited each trap with two septa, one loaded with 1 mg of poplar clearwing pheromone (96% pure, 4:1 ratio of (E,Z):(Z,Z)-3,13-octadecadienyl straight-chain 18 carbon alcohols, Cowles et al. 1996) and the other loaded with carpenterworm pheromone (9:1 ratio of Z3-E5-Tetradodecenyl Acetate, & E3-E5- Tetradodecenyl Acetate, Solomon et al. 1972, Doolittle and Solomon 1986). Pheromone-loaded septa for both lepidopteran species were purchased from Scenturion. Each week, trap catches of male clearwing and carpenterworm moths were recorded, a new sticky board was inserted, and septa were moved to the new insert. Trap capture at Potlatch was recorded from 10 July through 4 August 2001. An independent weekly trapping in Boise's plantation using 70 traps began 26 July and continued to 3 October 2001. Boise used delta traps baited with female clearwing sex pheromone only (Scenturion).

Monitoring traps were placed exactly in the center of each irrigation unit within Potlatch's plantation. This meant that regardless of eclosion site, the closest source of synthetic clearwing moth sex pheromone would be in the center of the irrigation unit from where the male emerged; thus there should be a correlation between the number of adult males captured and the population of western poplar clearwing moths within any particular irrigation unit.

Both companies switched from delta to bucket traps (Unitrap[®], Scenturion, Clinton WA) in 2002. Potlatch used 92 bucket traps baited with clearwing sex pheromone and another 92 baited with carpenterworm sex pheromone, whereas Boise utilized 70 bucket traps baited with clearwing sex pheromone, but Boise continued to use 70 delta traps baited with carpenterworm sex pheromone. All septa were loaded with 1 mg of sex pheromone of the respective species.

Type of trap and lures. Concerns about the accuracy of trap catches in 2001 in Potlatch's plantation, where septa containing sex pheromone for both moths were used in the same delta trap, led to follow-up experiments in 2004. We positioned six traps at the Sand Piper farm, all separated by >10 m, at 10 locations, each separated by at least 0.5 km. At each location, one delta trap was baited with clearwing sex pheromone, another with carpenterworm sex pheromone, and a third with both, identical to the strategy used in 2001. At each location, three-bucket traps were baited with the same pattern of lures. These 60 traps were monitored weekly from 2 June through 4 August 2004, thus overlapping the 2001 trap monitoring period.

Chemical control. Rather than following a recommended spray schedule (Bentley et al. 1994), Potlatch applied (2.34 L/ha) chlorpyrifos (Lorsban[®] 4E, Dow AgroSciences

LLC, Indianapolis, IN) aeri ally to an individual irrigation unit when trap-capture exceeded 100 moths per week in 2002. The type of clone in each unit sprayed was noted. OP-367 is a clone of *Populus deltoides* x *P. nigra*, whereas numbered clones (50-194, 50-197, 184-411, etc.) have a western cottonwood parent, *P. trichocarpa*. Of the fourteen days that applications of chlorpyrifos were made to control clearwing moths in Potlatch's poplar plantings in 2002, there were seven major efforts: 24 May (923 ha), 14 June (1,338 ha), 2 July (2,480 ha), 20 July (1,449 ha), 1 August (3,520 ha), and 28 August (1,537 ha). Boise used a minimal amount of chlorpyrifos, therefore its management strategy differed from that of Potlatch.

Damage assessment. A survey was conducted in December 2002 and these results were prorated over seven years for pulp, or 12 years for sawtimber rotation to estimate damage due to clearwing attack over the life of the tree. Another measurement of damage was the need to replant sapling damaged by western poplar clearwing moth larvae.

Endemic populations of clearwing moths. *Salix* and *Populus* species are native to the riparian areas parallel to these hybrid poplar plantings. In a few locations these endemic host trees are found adjacent to poplar farms. Pheromone-baited traps for both clearwing and carpenterworm moths were positioned in riparian habitat along the Columbia, Snake, Umatilla, and Walla Walla rivers. Trap capture was recorded weekly, male moths were discarded, and new septa were used each month.

Data Analysis. All experiments were analyzed using repeated measure analysis of variance (ANOVA), least significant difference (LSD) for multiple comparisons, and LSMEANS for interaction effects, by using PROC GLM (SAS 9.1). Significance levels

were set at a $P < 0.05$. Graphical representations of these data were made using SigmaPlot 9.01 (Systat Software Inc., Point Richmond, CA).

Results

Pheromone baited traps. Initial visual observations of insect damage in poplar plantations in September 2000 and Spring 2001 did not identify damage caused by clearwing larvae. A total of 95 male clearwing moths were captured between 3 and 26 July, an average of 0.26 (SD±0.88) moths per trap per week (moths/trap/week), $n=368$ (n =number of samples; where each sample equals one trap's catch of moths/trap/week). Between 26 July and 3 October 6,497 male moths were captured in Boise's plantation, an average of 15.14 (SD ±22.7) male moths/trap/week, $n=428$. The large standard deviation was indicative of spotty distribution of clearwing moths: 10% of the 428 trap samples captured 49% of the total catch, and on 82 occasions traps failed to capture any moths.

Over four weeks beginning 7 July 2002, 18,537 male clearwing moths were captured in 92 bucket traps in Potlatch's plantation (50.37 ± 3.84 moths/trap/week), a 196-fold increase over trap catches in the same number of traps, placed in the same locations, on the same mid-season dates in 2001 (Fig. 1). Likewise, trap capture in Boise's plantation from 24 July through 23 September 2002 was 15,841 male clearwing moths (25.14 ± 3.59 male moths/trap/week), a 2.44-fold increase in trap catch compared to a year earlier.

Boise's pheromone-baited traps captured 47,721 male clearwing moths between 17 April and 23 September 2002 (29.69 ± 2.02 male clearwing moths/trap/week) (Fig. 2). Clearwing populations were concentrated: Hill Top farm's traps captured 52.3 (± 6.27) male moths/trap/week over the entire season of 23 weeks, whereas traps positioned at the

Wallula farm averaged only 7.1 (± 1.6) male moths/trap/week over the entire season. Traps in Potlatch's plantation captured 60,710 male clearwing moths over 24 weeks of monitoring in 2002, an average of 27.50 (± 1.14) male moths/trap/week. As with Boise's capture record, the season long number of male moths captured in Potlatch's plantation varied considerably between traps (range 91.1 ± 8.86 down to just 0.79 ± 0.31 moths/trap/week).

Type of trap and lures. We repeated in 2004 the trapping method of using two septa containing pheromones for both clearwing and carpenterworm moths to examine their influence on trap catch records. Delta traps baited with one septum of clearwing pheromone captured an average of 13.2 (± 1.8) male moths/trap/week; whereas delta traps baited with both clearwing and carpenterworm moth pheromone captured an average of 9.6 (± 1.3) male clearwing moths/trap/week; thus there was no significant difference ($F=4.11$, $df = 1$, $P = 0.0525$) between having both lures in the same trap and having only the clearwing lure (Fig. 3).

Figure 1 shows that there was a significant difference ($F= 84.32$, $df =1$, $P < 0.0001$) between the delta traps used in 2001 and bucket traps used in 2002. Regardless of lure combination, delta traps caught (11.4 ± 1.12 moths/trap/week) significantly more ($F =18.14$, $df=1$, $P=0.0002$) male clearwing moths than bucket traps (5.2 ± 0.84 moths/trap/week) in 2004 (Fig. 3).

Chemical control. Both companies used broad-spectrum insecticides in 2002, either directly targeting adult clearwing moths or targeting other pests at a time that could have influenced adult clearwing populations. Potlatch applied Lorsban® on 14 different days to 74 irrigation units; some were sprayed 6 times, and one received 7 sprays, so that

altogether 213 unit/application dates were recorded and a total of almost 37,500 liters of chlorpyrifos were applied. Comparisons were made between trap captures at these sites over five wks, starting two wks before each application, the week after, and two wks after the application of an insecticide. Trap catches of clearwing moths were lower in irrigation units treated with Lorsban the week following the application but two-wks-post-application trap catches exceeded the capture recorded two wks before application (Fig. 4).

Possibly more important than the insecticide used was the host clone type. Twenty-four planting units of pure OP-367 were sprayed an average of 0.83 times. From these trees, 24.6% as many male moths were captured (mean \pm SE=227 \pm 35.5 moths) as were captured in the 29 units ((mean \pm SE=925 \pm 147 moths) consisting of varied numbered clones that were sprayed an average of 3.14 times. A smaller sample of ten mixed-clone stands, consisting of OP-367 and varied numbered clones, were sprayed 2.22 times and had an average season capture per unit of 595 \pm 113 moths, which was 2.62 times greater than the count found in pure OP-367 stands, but only 64% of the number found in planting units with varied numbered clones.

Another way to evaluate an effect related to the clone is through analysis of male moth capture rate at the pumping station in the center of each irrigation unit. If trap capture of male moths in each of Potlatch's irrigation units were grouped for the entire season, traps in 44 units captured <500 moths, another 22 trap units captured >500 and <1,000, and 26 traps in other units captured >1,000 moths. The 44 irrigation units where <500 male moths were captured for the entire season were predominantly (84%) clone OP-367, whereas 69% of those 26 planting units where >1,000 male moths were captured

had no OP-367 clones planted. Age of the trees had no apparent correlation with the number of male western poplar clearwing moths captured; the average age of trees (\pm SE) in the 44 planting units where <500 moths were captured was 4.9 ± 1.7 years, and in those stands where traps captured >1,000 the average age was 4.2 ± 2.5 years old.

Damage assessment. We surveyed 400 three-year-old trees at Boise's Wallula farm on 10 December 2002; 177 (44.2%) of those trees had been damaged by clearwing larvae. Assuming that those damaged trees were injured evenly over the previous three seasons, 59 were damaged each season. This would equal an attack rate of 14.75% a year. If this rate were projected forward to seven or 12 years, the earliest these trees could be harvested for pulp or sawtimber, it would suggest a damage level of 69% or 86% would occur, respectively.

Perhaps a more serious damage problem is the loss of newly planted trees to the girdling attack of western poplar clearwing moth larvae. Of the 459,349 newly planted trees in Potlatch's plantation, 18,995 (4.1%) needed immediate replanting, another 8,056 (1.75%) required a second replanting, and a third replanting replaced 8,680 (1.89%) of the saplings girdled by western poplar clearwing moth larvae.

Endemic populations. In riparian zones only 8% of the wood boring moths (Sesiidae and Cossidae) captured in traps baited with species-specific sex pheromone were male clearwing moths, whereas in poplar plantations these Sesiidae moths represented 95% of the moths captured in pheromone baited traps.

Discussion

Outbreak of an endemic population. Large-scale commercial hybrid poplar production was initiated in 1991 to provide a year-round supply of short rotation pulp-

sized trees. Center-pivot irrigation units were leased or bought, and water delivery systems were converted to drip line irrigation. What were once irrigated potato, onion, or alfalfa fields were converted to rapidly growing poplar trees. Unfortunately these plantations were located close to native riparian zones. Our research suggested that the western poplar clearwing moth is normally found in low numbers in natural riparian zones compared to another potential Lepidoptera pest, the carpenterworm. Unmanaged mixed stands of *Populus* and *Salix* species of various ages can be found in narrow bands along permanent waterways in arid eastern Oregon and Washington. Older and sometimes weaker trees are ideal for carpenterworm populations, but western poplar clearwing moths prefer younger, more rapidly growing trees. These pheromone baited traps placed in riparian zones caught male carpenterworm moths in a much higher proportion to male clearwing moths when compared to trap catches of these two moths in the poplar plantations. A decade later, an insect species that was not even listed as a pest of poplar trees (Chastagner and Hudaki 1999, Coyle et al. 2005) became an economically important pest to poplar plantings in the Columbia Basin in a period of just three years. Overall, 2002 was clearly an outbreak year for clearwing moths in both Boise's and Potlatch's plantations. Our data shows how an endemic population of a once non-pest species moved from a natural habitat into a monoculture of a preferred host plant. Western poplar clearwing moth achieved pest outbreak status within a very short period of time. Episodic outbreaks of forest pests are common cyclic events and many models have been proposed (Dwyer et al. 2004); our brief decade-long record of pests invading poplars planted in Washington and Oregon cannot exclude the possibility of a natural cycle repeating itself over time. Even in natural forest there are outbreaks of endemic

pests still being documented for the first time (Stephen et al. 2001), and with time the record may show a cyclic nature to these pests, too.

The initial report of a dramatic 190-fold increase in western poplar clearwing moth population in Potlatch's plantation between 2001 and 2002 (Fig. 1) was suspect because we used delta, rather than bucket, traps and loaded the traps with the sex pheromones of two species. Perhaps clearwing males were less attracted to their conspecific sex pheromone if the sex pheromone of the carpenterworm moth was also present. Multiple sex attractants loaded into individual traps reduced the capture of coneworm adult males of *Dioryctria amatella* (Hulst) in conifer seed orchards (DeBarr et al. 1983). However, when different combinations of trap design and pheromone bait were used in 2004, there was no significant difference between the number of male clearwing moths captured in delta traps containing clearwing pheromone only (13.2 ± 1.08 moths/trap/week; n=10), compared to those baited with both clearwing and carpenterworm sex pheromone (9.6 ± 1.33 moths/trap/week; n=10). More surprising was the greater efficiency of the delta traps compared to bucket traps (6.4 ± 1.55 moths/trap/week; n=10) when each was loaded with only a clearwing moth lure (Fig. 3). When trap designs were compared disregarding how they were baited (clearwing pheromone only or pheromone for both species), delta traps caught significantly more male clearwing moths (11.4 ± 1.12) than bucket traps (5.2 ± 0.84). This would suggest that delta traps loaded with pheromones of both species were accurately reporting the low population of clearwing moths in Potlatch's poplar plantation in 2001. The possibility that the bucket traps used in 2002 may have underestimated the western poplar clearwing moth population the following year is alarming.

Type of trap and lures. Lepidopteran trapping efficacy has been compared among many trap types (Gray et al. 1991, Lopez 1998, Mullen et al. 1998, Athanassiou et al. 2004). In forest systems, Asaro et al. (2004) reported greater numbers of Nantucket pine tip moth *Rhyacionia frustrana* (Comstock) captured in wing than delta traps. More importantly with respect to clearwing moths, Rocchini et al. (2003) reported no significant difference between wing and green Unitraps (Phero Tech, B.C. Canada) in capturing the Douglas-fir pitch moth, *Synanthedon novaroensis* (Henry Edwards) (Sesiidae). He did not include delta traps in his trial.

Documenting nocturnal moth behavior requires night-vision goggles (Lingren et al. 1980, Goodenough et al. 1989), harmonic radar (Riley 1989, Svensson et al. 2001), or laboratory wind tunnels under infra-red illumination. Observing the diurnal behavior of male clearwing moths approaching bucket traps provided anecdotal evidence that males are often reluctant to enter these traps. Monitoring traps over 3 seasons involved >6,000 day-time observations. We frequently saw males hovering downwind of the bucket traps, some making repeated approaches toward the trap, often entering the space above the funnel, and then retreating. Delta traps with sticky surfaces may prevent moths from retreating once they have entered the trap. Perhaps this advance-and-retreat activity is a common behavioral reaction of males to a high concentration of sex pheromone within the trap. Our research suggests that properly managed delta traps can be more efficient than bucket traps under ideal weather conditions and low pest pressure (Fig. 3). Management must include replacement of sticky inserts weekly and changing of the pheromone septa monthly. Dry weather conditions can result in dust accumulation on the sticky inserts rendering them useless within several days, thus bucket traps are more

reliable for season-long trapping of western poplar clearwing males. Also the delta trap sticky surface can become saturated when more than 100 moths per week are trapped, whereas we have trapped up to 584 male moths/bucket trap/week in 2002.

Pheromone baited traps. The direct comparison (Fig. 2) of male clearwing moths captured in 2002 in Boise and Potlatch plantations was quite informative. The two plantations were approximately the same size: Boise had 6,200 ha and Potlatch had 6,700 ha in production in 2002. The number of moths captured per trap was significantly different ($P < 0.05$) on three of the 21 wks (Fig. 2). The season long difference in total moths captured in each company's plantation could have been simply a difference in the number of traps deployed; Boise used 70, while Potlatch deployed 92. Over the entire season Boise's trap capture averaged 29.69 male clearwing moths/trap/week and Potlatch's trap capture rate was 27.50 male moths/trap/week. If Boise's season-long capture record included a proportional trap catch for 22 additional traps, that amount would equal 62,719 moths, a number only 3% greater than Potlatch's season-long capture record of 60,710 moths.

Pest management practices at the two companies were quite different in 2002. Bentley et al. (1994) recommended using chlorpyrifos every two wks to control western poplar clearwing moths in California; Potlatch modified this and only treated irrigation units where monitoring traps captured >100 moths per week. Thus some units were not sprayed, and others received between 1 and 7 applications during the season, depending on pest pressure. Boise sprayed 365 ha with chlorpyrifos, only 7% of the area sprayed by Potlatch, and yet the two lines representing trap capture over separate plantations were almost identical. High capture rates (wks 2, 5, 8, 11, and 13) and low capture rates (wks

3, 6-7, 9-10, 12, and 14) of male moths for all farms were very similar, whether contiguous or separated by >65 km, and under different management practices. Adult eclosion patterns could be related to local weather conditions and some of the low trap numbers followed weeks with several nighttime temperatures below 10°C. Cool nights are known to reduce dispersal flights of wood-boring insects (Hanks et al. 1998). The biology of the overwintering clearwing population, however, is probably more important than are weather fluctuations. We sampled poplar logs throughout the winter for several seasons and all immature stages, including pupae, were found in mid-winter. Thus we suspect the eclosion of male moths represented in Fig. 2 beginning in late April and early May and continuing until early August is from overwintering larvae, whereas overwintering females emerging in April and May could have mated and oviposited eggs that matured into males captured in late August through September. This would partially explain the large population detected in Boise's plantation after August 2001.

Chemical control. Chlorpyrifos has been widely recommended for control of clearwing moths (Bentley et al. 1994, Bergh and Leskey 2003, Riedl et al. 1985, Kain and Straub 2001). In orchard or urban situations chlorpyrifos is applied directly to the bark. The half-life of chlorpyrifos residues in an orchard was reported to be <1 day (Knight and Hull 1992); on elm the half-life is between 1.1 and 2.9 days (Jin and Webster 1997). The value of chlorpyrifos must therefore be in its persistence when in contact with soil, where its half-life exceeds 50 days (Bulinski and Matthiessen 2002). Soil treatment with chlorpyrifos (All et al. 1985) killed both eggs and neonates of the grape root borer, *V. polistiformis*. Other efforts to spray insecticides (chlorpyrifos and lindane) against clearwing pests have failed (Yonce 1982), and suggestions of injecting the chemicals

(endosulfan, lindane, diazinon, carbaryl, and *Bacillus thuringiensis*) directly into galleries (Solomon 1985) would be impractical for the ~19 million hybrid poplar trees grown in Oregon and Washington.

The use of broad-spectrum insecticides in 2002 eliminated any hope for biological control agents to have had an effect on clearwing larvae, as reported for other Sesiidae species from Canada (Cossentine et al. 1990) and eastern Europe (Georgiev 2001 a, b; Kahounova and Mracek 1991). Our light trapping of nocturnal moths and pit-fall trapping of ground-active beetles indicated a dramatic decline in insect fauna after several applications of chlorpyrifos.

With the failure of contact insecticides to control western poplar clearwing moth populations, we're investigating the possible short-term solution of broadcast spraying of sex pheromone to disrupt mating of moths. A longer-term solution will probably involve selection of less preferred host plants. Currently much of the plantings are hybrids of two endemic species to North America: *Populus trichocarpa*, the western black cottonwood, and *P. deltoides*, the eastern cottonwood. Clones with *P. trichocarpa* are the most susceptible to western poplar clearwing moth attack, whereas clones from European (*P. nigra*) or Asian (*P. suaveolens* Loud (Syn. *P. maximowiczii* Henry) rootstock are attacked less. Breeding for pest resistance in poplars is in its infancy, but harbors great potential in that a wide diversity of tree species exists. Weisgerber and Han (2001) for example, reported 17 species of poplar from one region of Tibet alone, and they highlight the diversity of *Populus* found in China.

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Figures:

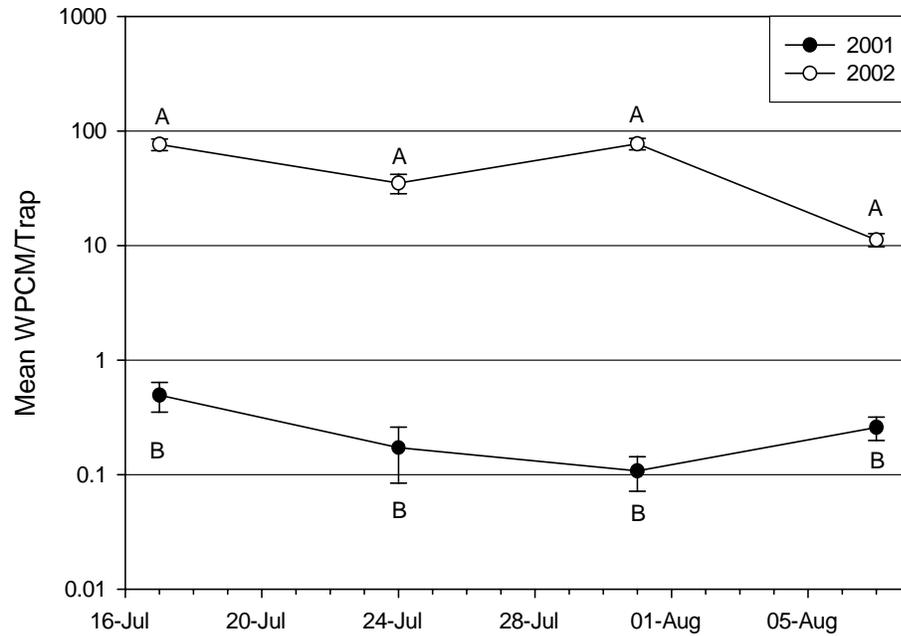


Figure 1: Logarithmic representation of the number of male western poplar clearwing moths (WPCM) captured in a 4-wk period in 92 traps positioned at each pumping station over Potlatch’s $\approx 6,620$ ha. These two lines compare the 2001 mean capture of 0.26 ± 0.13 (SE) moths per trap per week to the 2002 mean capture of 53.5 ± 26.75 moths per trap per week. Means with the same letters are not significantly different ($P < 0.05$).

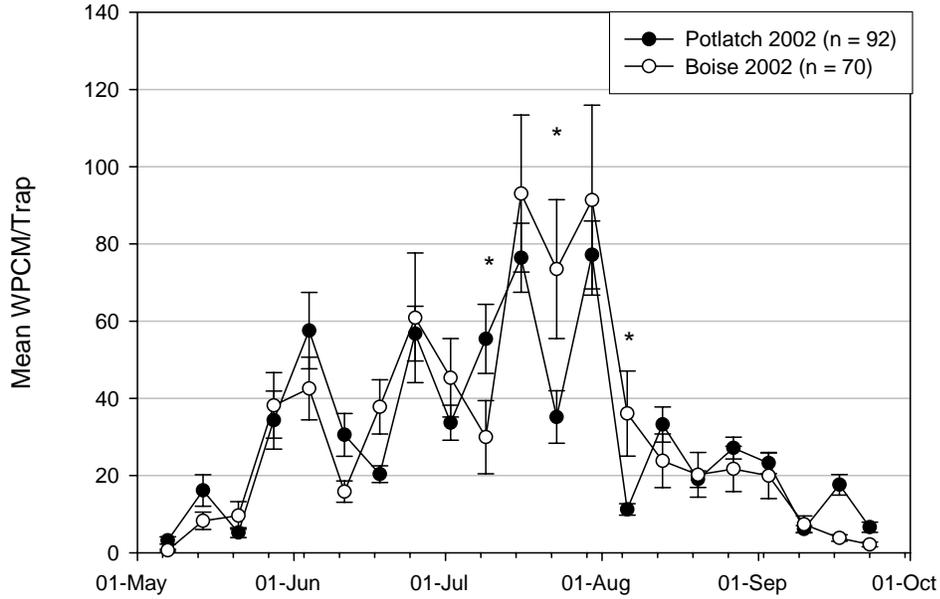


Figure 2: Direct comparison between Boise’s and Potlatch’s trap capture of male western poplar clearwing moths in 2002. Both lines represent a season-long survey of $\approx 6,475$ ha for each beginning in late April and ending in early October. Boise’s 70 traps captured 47,721 moths, whereas Potlatch’s 92 traps captured 60,710 moths over the entire season. Asterisks (*) represent dates where the mean trap captures between the two farms are significantly different ($P < 0.05$).

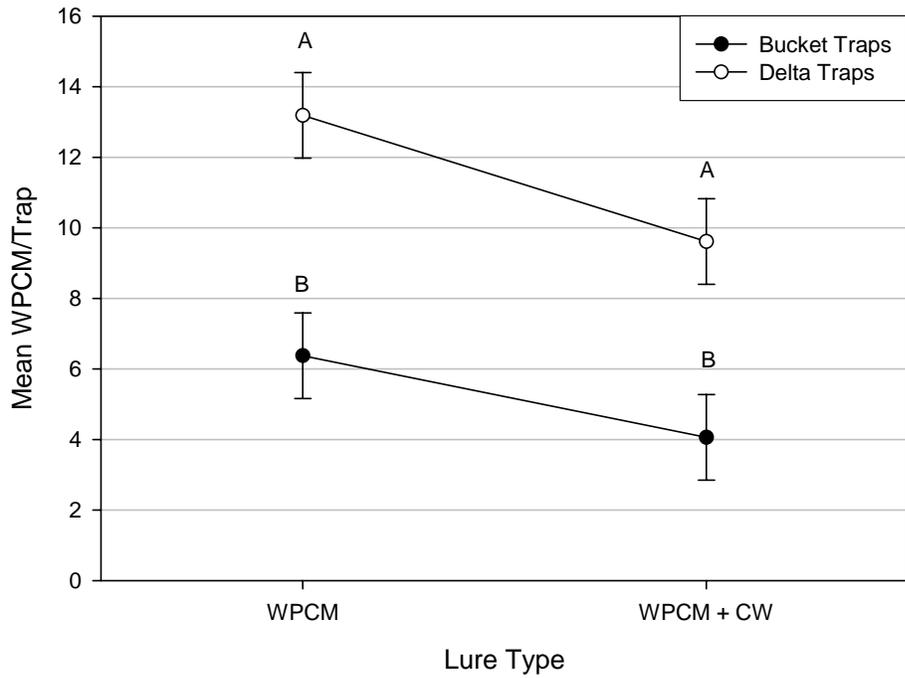


Figure 3: Mean number of male western poplar clearwing moths (WPCM) captured in bucket or delta traps baited with two septa (WPCM+CW), one with 1 mg of WPCM pheromone and another with 1 mg of carpenterworm moth (CW), compared with traps containing only a 1 mg of WPCM pheromone lure. Mean with the same letters are not significantly different ($P < 0.05$).

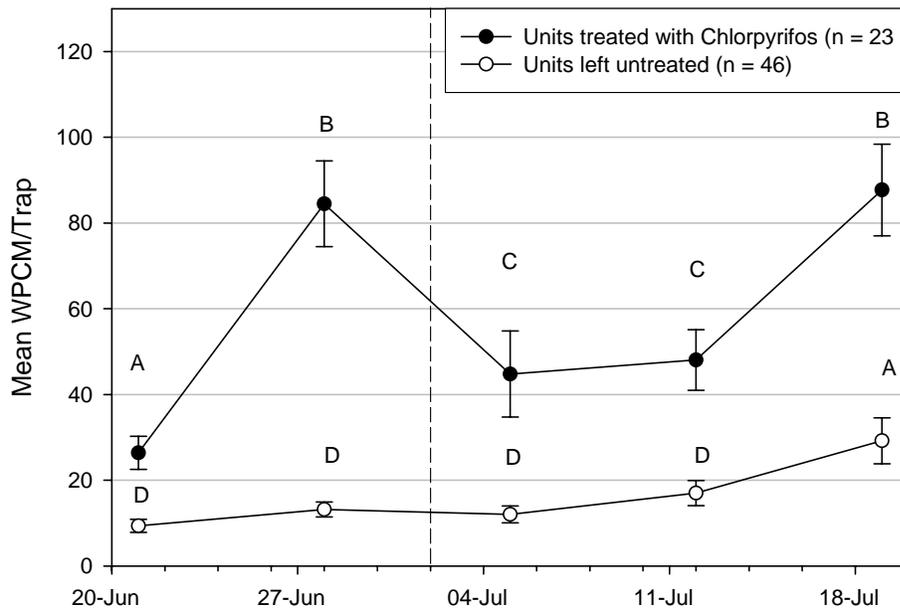


Figure 4: Comparison of the change in trap capture over time in 23 units treated with chlorpyrifos to 46 other units that were not treated with any pesticides. This graph represents trap catches 2 and 1 wk before treatment, the week of treatment on 2 July (vertical dotted line), and 1 and 2 wk after chlorpyrifos application. No chemical control was used in the untreated units within this 5-wk period. Means with the same letter are not significantly different ($P < 0.05$).

**Chapter 4: A pheromone-based strategy to control the western poplar clearwing moth
(Lepidoptera:Sesiidae) in hybrid poplars.**

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ABSTRACT: We have taken a laboratory discovery of a sex pheromone (Cowles et al., 1996) to a registered biopesticide (EPA Reg. No.56336-40) in 10 years. A pilot test using multiple septa loaded with western poplar clearwing moth *Paranthrene robiniae* (Hy. Edwards) sex pheromone in 2002 suggested a pheromone-based control strategy would shut down the capture of male moths in pheromone-baited traps. The following year, three concentrations of an aerially applied flowable formulation and a membrane pheromone dispenser system were tested beginning 1 June. Each concentration and formulation resulted in immediate shutdown of traps baited with 1 mg lures over 2,948 ha of poplars. Trap capture of male moths in super traps baited with 10 mg lures was inversely proportional to the amount of pheromone and formulation applied to each unit. However, damage from *P. robiniae* continued. Delayed application of pheromone allowed overwintering moths to mate, and damage from their progeny resulted in a significantly increased need for second and third replanting of lodged saplings. A regression analysis of *P. robiniae* larval head-capsule widths estimated hatch occurred before 7 June 2003 and significantly more damage was located at the base of the newly planted trees than above 6 cm. Both observations suggest mating occurred prior to pheromone application.

In 2004, 12,991 ha of poplars were treated with *P. robiniae* pheromone, damage to sentinel trees was significantly ($P = 0.0005$) reduced in one plantation (6,710 ha), and reduced, but marginally insignificant ($P = 0.057$) in the other plantation (6,281 ha). There was a significant reduction in the need for second and third replanting in 2004 compared to 2003.

Economic considerations led to modified strip spraying and border spraying strategies in 2005. Both strategies shut down trap catches. Damage to sentinel trees continued to decline where strip spraying was implemented, but damage to sentinel trees increased in areas where border spraying was used.

All commercially grown poplars in eastern Oregon and Washington are currently being protected from *P. robiniae* attack by using a pheromone-based male confusion strategy.

KEYWORDS: male confusion, mating disruption

Introduction

Three timber-producing corporations, Potlatch (Boardman, OR), Boise (Wallula, WA), and GreenWood Resources (Portland, OR) have established plantings of hybrid poplar on approximately 14,164 ha (35,000 acres) in eastern Oregon and Washington. Most of these trees will be harvested for pulp, but because of declining pulp prices, one company (Potlatch) has made a market-based decision (Stanton et al., 2002) to redirect its management practices toward the production of non-structural sawtimber. Western poplar clearwing moths *Paranthrene robiniae* (Hy. Edwards) (Lepidoptera:Sesiidae) damage trees by burrowing as larvae into the bole or limbs of trees, compromising the structural integrity such that moderate to high winds cause limbs and trunks to break. Besides damage to mature trees, newly planted saplings attacked by *P. robiniae* easily lodge. Late replanting is essentially impossible, as the adjacent trees proceed to close the canopy at a uniform rate and any newly planted “sticks” are unlikely to survive. Depending upon the crop’s intended use, pulp or timber, this vacant space within the planting constitutes a seven- to twelve-year loss in production.

Initial monitoring of male *P. robiniae* response to the female sex pheromone began in 2001. One pheromone-baited trap was placed in each planting unit of ~65 ha. Few male moths were captured in 2001 (0.26 ± 0.88 *P. robiniae* per trap per week) in mid-season, but in 2002 the mid-season numbers increased dramatically (50.37 ± 3.84 *P. robiniae* per trap per week), constituting an outbreak year (Brown et al., 2006).

Bentley et al. (1994) recommended the application of Lorsban® (chlorpyrifos, 2.23 kg active ingredient/hectare [ai/ha]) every two weeks to control *P. robiniae* in California. Potlatch modified Bentley’s recommendation, spraying only those irrigation

units where 100 or more male moths were captured in pheromone-baited traps the previous week, rather than spraying by date. This strategy of using broad-spectrum insecticides failed in 2002 (Brown et al., 2006), so we investigated the possible use of a pheromone-based strategy to resolve the problem.

Sesiid moths are known to be susceptible to a pheromone-based strategy. Earlier reports by Gentry et al. (1980) and Snow et al. (1985) of successful pheromone strategies to control the peachtree borer *Synanthedon exitiosa* (Say) and lesser peachtree borer *S. pictipes* (Grote and Robinson), and the report by Johnson et al. (1991) on the use of pheromones to control low to moderate populations of grape root borer *Vitacea polistiformis* (Harris) suggested the same strategy should work against *P. robiniae*. However, these early reports of success were based on reduced trap capture of male moths, assuming that males could not find females if they were not captured in traps loaded with mg amounts of sex pheromone. An application targeting the lesser peachtree borer with (E,Z)-3-13-octadecadien-1-ol acetate (ODDA), the sex pheromone of *S. pictipes* (Tumlinson et al., 1974), corroborated trap shutdown or male confusion in orchards permeated with the sex pheromone, and a reduction of the pest infestation was noted (Pfeiffer et al., 1991). Later, Pfeiffer and Killian (1999) used a 96:4 blend of (Z,Z)-3,13-ODDA and (E,Z)-3,13-ODDA, the best formulation for the peachtree borer in a small 2-ha plot of apples. Although pure (Z,Z)-3,13-ODDA is the most effective sex attractant for the dogwood borer *S. scitula*, and Karandinos et al. (1977) reported any addition of the E,Z isomer reduced the male's response to the lure, Pfeiffer and Killian (1999) still reported shutdown of trap catch of *S. scitula*, but damage was not significantly reduced. Thus these earlier reports of male confusion where the number of

male moths captured in pheromone-baited traps were shut down does not necessarily constitute mating disruption, wherein a reduction in pest population or damage should also be evident.

A series of experiments were developed and initiated to determine whether a pheromone-based strategy targeting adult *P. robiniae* males might have promise. Here we report results from a pilot experiment in 2002 that demonstrated concentrated release of pheromone had potential to shut down trap catch. This was followed with applications of sex pheromone at three different rates of flowable and one rate of hand-applied membrane dispensers to 2,948 ha of poplar beginning in June 2003. We conducted baseline damage surveys before and after pheromone application to document whether we had managed to suppress damage from *P. robiniae* attack.

Full-season pheromone protection was implemented on 12,991 ha in 2004. Only two small, isolated farms, Wallula (460 ha, Wallula, WA) and Sandpiper (1,200 ha, near Patterson, WA) were not treated with *P. robiniae* pheromone. Besides weekly trap capture records of male *P. robiniae* moths, we monitored over 4,000 sentinel trees for clearwing damage and documented loss of new plantings attributed to *P. robiniae* damage.

Successful reduction of *P. robiniae* populations in 2004 and fiscal concerns resulted in poplar pest managers deciding to spray pheromone in strips or on borders in 2005, rather than covering all poplar trees each month. Changes in ownership of poplar plantations increased the holdings of Greenwood Resources and reduced those of Boise in 2005.

We are reporting the effective reduction in trap capture of male *P. robiniae* moths after utilizing a pheromone strategy for 3 sequential years, as compared to spraying chlorpyrifos in 2002 (Brown et al., 2006). We are documenting the suppressed damage to poplar trees from *P. robiniae* attack. The commercial pheromone formulation Checkmate WPCM-F received full registration in 2006 and commercial poplar growers in WA and OR have adopted a pheromone-based control strategy for *P. robiniae*. We are confident in our recommendation of using 2.47 g/ha of sex pheromone divided into six applications to suppress damage from *P. robiniae*.

Material and Methods

Plantations. Throughout this manuscript we have consistently used several terms to describe an area of poplar trees. We used the term *plantation* to describe the entire holdings of one company within a region, e.g., Boise's, GreenWood Resources' or Potlatch's plantations in northeastern Oregon. The term *farm* refers to the largest subdivision of a plantation, e.g., Boise's Wallula farm, Potlatch's North and South farms, or GreenWood Resource's Sand Lake and Sandpiper farms. *Irrigation unit* is used to denote a managed parcel of land irrigated by a common pump and planted at the same time; these are usually between 64.75 (160 acres) and 113.3 (280 acres) hectares in size. A *planting unit* is a smaller portion of an irrigation unit and we reserved the term *block* to describe our experimental plots.

All plantations are grown on sandy soils; originally this area was high desert containing sage (*Artemisia* spp.), rabbitbrush (*Chrysothamnus* spp.) and related vegetation. Trees are planted 1,285 trees per ha for pulp and 717 trees per ha for sawtimber and provided with fertilizer through drip irrigation lines operated 10 to 24

h/day depending upon tree age and time of year. Hybrid poplar clones are of *Populus trichocarpa*, Torr. and Gray, *P. deltoides*, Bartr. and Marsh. and *P. nigra* L. parentage (Salicaceae). Trees planted in April can exceed 5 m in height by October and at harvest after 12 years can exceed 35 m. We will refer to newly planted trees as first-year trees, one-year-old trees as second-year trees and so on, based on their age at the end of the growing season.

Trapping Method. *Monitoring traps.* All traps used for monitoring were Unitrap® bucket traps baited with *P. robiniae* sex pheromone purchased from Suterra LLC (213 SW Columbia, Bend, OR). The synthesized pheromone blend consisted of a 4:1 ratio of (E,Z):(Z,Z)-3,13-octadecadienyl alcohols (ODDOH) (Cowles et al., 1996). Each trap also included an approximately 2 cm² piece of Vapona® (dichlorvos) No-Pest Strip (Monsanto Canada Inc, Mississauga, Ont.) to kill the trapped moths. All bucket traps were thoroughly cleaned before deployment. Each trap was monitored weekly, at which time the number of male *P. robiniae* were recorded and the traps were emptied. Lures and killing strips were replaced every other month.

Super lure traps. Traps baited with 10 mg of *P. robiniae* sex pheromone were also used to monitor male flight activity in areas saturated with pheromone. Three bucket traps baited with 10 mg lures were positioned in a transect running northwest to southeast, perpendicular to the prevailing wind direction inside each experimental block in 2003. In 2004 and 2005 only one 10 mg baited trap per unit was used, and these were centrally positioned near the 1 mg monitoring traps. Each 10 mg baited trap was also monitored weekly, with the number of male *P. robiniae* recorded and the traps emptied. Lures and killing strips were replaced every other month.

Pilot Experiment. An experiment to estimate the potential for a pheromone-based strategy was initiated in August 2002. Plastic netted bags (10 x 25 cm) (Quadel Industries, Inc., 200 Troy Street, Coos Bay, OR), each containing a septum loaded with 1 mg of female *P. robiniae* sex pheromone, were stapled to trees on 14 August 2002. One septum loaded with 1 mg of sex pheromone on every tree was considered a full rate (1.29 g/ha); a septum on every other tree was considered a half-rate (0.64g/ha); on every fifth tree, 1/5 rate (0.26 g/ha); and on every tenth tree, 1/10 rate (0.13 g/ha). A separate unit without added pheromone septa was retained as a control. Each experimental unit contained 400 trees, 20 rows of 20 trees, a square approximately 0.311 ha, and all were located at least 500 m apart. The prevailing winds were from the southwest toward the northeast, so to avoid possible drift of pheromone influencing these results the full-rate test site was positioned downwind of the 1/2-rate, 1/5-rate, 1/10-rate, and control blocks, respectively. In the center of each block of 400 trees was one bucket trap baited with a 1 mg standard septum and another baited with a super lure loaded with 10 mg of *P. robiniae* sex pheromone. These pheromone-baited monitoring traps in each block were monitored daily for four days before insertion of septa representing various rates of pheromone. Once the added septa were in place, these traps were monitored daily for one week and then weekly for six more weeks.

Pheromone Formulations. *Flowable.* The flowable pheromone used in the pheromone control strategy was manufactured by Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan) and formulated by Suterra LLC (213 SW Columbia, Bend, OR). The flowable pheromone formulation was 14.02% (E,Z)-3,13-Octadecadienol, 3.55% (Z,Z)-3,13-Octadecadienol and 82.43 % inert ingredients, which includes the microcapsules. The

flowable pheromone came in 581 ml bottles with a concentration of 0.172 g ai/ml. The flowable pheromone was applied in 46.8 liters of water with 1.16 liters of Nu-Film 17® (Miller Chemical and Fertilizer, Adelaide, AU) per hectare.

Manufacturer recommendations suggested that the flowable formulation be applied every 30 days for optimal effectiveness. To validate the manufacturer's recommendation we set up a field experiment to verify the flowable pheromone's longevity. Concentrated flowable pheromone was diluted so 1 ml was equivalent to 25 mg (1.46ml of concentrated flowable pheromone in 8.54ml of water). One (1) ml (25 mg) of diluted flowable pheromone was loaded onto filter paper and used as a lure for *P. robiniae* in bucket traps. We had replications in four independent fields and the trap catches were recorded for nine consecutive weeks.

In 2003, the flowable pheromone was applied four times, with the first application made 1 June and subsequent applications made monthly. We had three application rates of the flowable formulation in 2003: 7.41, 2.47, or 1.24 grams ai per hectare per season (g/ha/season) (Table 1). For each monthly application, one fourth (1/4) of the total assigned treatment was applied. In 2003, only first- and second-year trees were treated with pheromone applications (2,948 ha).

All poplar plantations (12,991 ha) except the Sandpiper farm (1,173) were treated with pheromone in 2004. First- and second-year trees were treated with 2.47 g/ha/season and third-year and older trees were treated with 3.7 g/ha/season. Applications began 1 April with five subsequent applications made monthly. The 3.7 g/ha/season treatments were split into six equal monthly applications of 0.62 g/ha. First- and second-year trees received applications of 0.309 g/ha in April, June, August, and September, with another

0.618 g/ha applied by air in May and July, together these are equivalent to 2.47 g/ha/season.

The 2005 description of flowable pheromone usage will be discussed on a landholder basis because of some restructuring of land ownership between companies.

Potlatch Corporation continued with pheromone coverage of their entire area. Pheromone treatments were made in six monthly applications starting 1 April. First- and second-year trees received 2.47 g/ha/season in six equal applications. Third-year trees and older also received the 2.47 g/ha/season treatment however, only one third of each irrigation block was treated in any given month; we have called this *strip spraying*. An irrigation block is split into six sections (1, 2, 3, 4, 5, & 6). In April and July sections 1 and 4 were treated. In May and August sections 2 and 5 were treated, and in June and September sections 3 and 6 were treated.

Greenwood Resources treated only units with first- and second-year trees plus a 55 m buffer around these young trees. These units were treated with six equal monthly applications that totaled 1.85 g/ha/season.

Membrane Formulation. Membrane dispensers were also made by Suterra LLC (213 SW Columbia, Bend, OR). In 2003, six blocks received membranes (Table 1) formulated with 24 mg of *P. robiniae* sex pheromone. Membranes were applied once, at an interval of one membrane on every tree in every third row or one membrane on every tree in every fifth row depending on if the trees were spaced for timber or pulp, respectively; each is equivalent to 7.41 g/ha/season. In 2004, membranes were loaded with 40 mg of pheromone and applied to every tree in every seventh row (3.95

g/ha/season) in 27 units of trees managed for timber. Membrane dispensers were not used in 2005.

In 2003, samples from membrane dispensers were collected from the field and submitted to Suterra LCC to determine the amount of pheromone remaining. Membranes were placed in the field 1 June 2003. Samples were taken 8 July, 1 August, 2 September, and 6 October of 2003.

Damage Assessment. *Units Surveyed.* Each year all units with first- and second-year trees were used for damage assessment. These trees were split into approximately 65 ha experimental blocks. The number of experimental blocks included in our annual damage surveys depended upon the acreage planted by the growers. In 2003 we had 60 blocks, in 2004 we had 72 blocks, and in 2005 we had 45 survey blocks.

Of the 60 blocks surveyed in 2003, 14 were control blocks (935 ha) that received no pheromone treatment (eight of the control blocks were first-year trees and six were second-year trees), 15 (11 first-year and 4 second-year) blocks (1,046 ha) received 1.24 g/ha/season of pheromone, 19 (12 first-year and 7 second-year) blocks (1,174 ha) received 2.47 g/ha/season, six (3 first-year and 3 second-year) blocks (366 ha) received a 7.41 g/ha/season; and six (6) first-year blocks (362 ha) received membranes equivalent to 7.41 g/ha/season (Table 1). In 2004, 65 of the blocks (3730.5 ha) were treated with 2.47 g/ha/season and 7 blocks (480.2 ha) were held as controls. In 2005, all 45 blocks (2522.8 ha) were treated with 2.47 g/ha/season.

Damage Surveys. New damage was assessed by surveying first- and second-year trees after leaf fall at the end of each growing season. Third-year trees and older were omitted from the damage survey because of their size. A predetermined sample of thirty

(30) trees in each experimental block served as sentinel trees; these were split into six groups of five-tree-row plots. The six groups were arranged so that they were evenly spaced in a diagonal transect across the unit going from the northwest to southeast so as to be perpendicular to the prevailing wind direction. Starting from the northwest corner of a unit, the first set of five sentinel trees were 3 rows east and trees number six through ten toward the south. The second set was 53 rows east of the northwest corner and trees numbered 51 through 55 toward the south, and the third set was 103 rows east from the northwest corner and trees number 101 through 103 towards the south. Sentinel tree sets four through six were found in the same manner but in reverse direction starting from the southeast corner of the unit. The entire sentinel tree was examined for damage. Damage to a tree will be referred to as a *hit* and consists of a *P. robiniae* larval entrance hole with frass actively being extruded.

Replant Data. Potlatch Corporation retains accurate records of how many trees they plant and replant every year. Every growing season about 9 to 13 units (600 to 800 ha) are planted with new trees. Each season multiple replanting efforts are needed and the number of planting sticks replanted is closely monitored.

Paranthrene robiniae larvae were extracted by hand from newly planted lodged trees from 1 July to 6 August 2003. Head capsule widths were measured using a dissecting scope with an in ocular micrometer (15x). Magnification on the scope was set at 2x, where 20 units on the micrometer equaled 1 mm.

Statistical Analysis. Assessment of monitoring trap shutdown (1 mg septa) and super lure trap capture (10 mg septa) were Log base 10 transformed for a normal distribution and analyzed using repeated measures ANOVA with LSMEANs for

assessing interaction effects between time and mean trap counts. The mean number of hits on 30 sentinel trees in each unit was used for assessment of damage (SAS, 2003). Pheromone treatment effects related to the damage survey were analyzed by ANOVA using LSDs for multiple comparisons and LSMEANs for assessing interaction effects between ages of the trees and year (SAS, 2003). Differences between the locations of damage on first-year trees were analyzed using repeated measures ANOVA and LSMEANs for interaction effects between damage location and year of survey (SAS, 2003). Data from the flowable pheromone longevity test was Log base 10 transformed to get a normal distribution and equal variance. Non-linear regression for the analyses of head-capsule width of *P. robiniae* larvae extracted from lodged trees was performed in SigmaPlot 9.01 (SigmaPlot, 2004). All graphical representations were made using actual means \pm standard errors with SigmaPlot 9.01.

Results

Pilot Experiment 2002. All experimental rates of *P. robiniae* sex pheromone resulted in immediate trap shutdown. The full rate, prior to septa placement, had a mean trap capture of 52.75 ± 25.47 (mean \pm SE) *P. robiniae* males per trap per day and after placement of a septum on every tree, the mean was reduced to 0.29 ± 0.31 *P. robiniae* per trap per day. The half rate had a mean capture of 72.25 ± 66.44 *P. robiniae* per trap per day before placement and 0.14 ± 0.15 *P. robiniae* males per trap per day after septa insertion. The one-fifth and one-tenth rates had means of 12.75 ± 2.88 and 20.25 ± 20.33 *P. robiniae* per trap per day, respectively, prior to septa placement and means of 0.28 ± 0.2 and 2.0 ± 1.33 *P. robiniae* males per trap per day after septa placement. The control block had the lowest capture rate, with a mean of 1.25 ± 0.86 *P. robiniae* per trap per day

prior to septa placement in test areas downwind; this was the only area where trap capture increased after placement. Trap capture in the control block was 1.71 ± 1.01 *P. robiniae* per trap per day after septa placement in the other experimental blocks.

Trap Shutdown 2003. *Monitoring traps.* Our 1 mg monitoring traps in treated units were essentially shut down in 2003 within seven days of the first pheromone application, which is shown by the significant date by treatment interaction effects ($F = 3.93$, $df = 46$, $P < 0.0001$). Pheromone application began on 1 June 2003. Trap catches in 17 units slated for pheromone treatment were significantly higher than in 76 untreated units (Fig. 1) for two weeks prior to commencement of pheromone treatment. For the weeks of 19 and 26 May, mean trap capture in units scheduled to be treated with pheromone were 98.88 ± 22.63 (mean \pm SE) and 73.88 ± 30.47 *P. robiniae* males per trap, respectively, while the mean trap capture in untreated units for those same weeks were 17.32 ± 4.73 and 18.67 ± 4.83 *P. robiniae* per trap, respectively. After pheromone application, the highest trap capture for any week within the treated units was that of 30 June with a mean of 0.94 ± 0.47 *P. robiniae* males per trap, significantly ($P < 0.0001$) fewer than the mean of 45.30 ± 17.86 *P. robiniae* males per trap captured in untreated units the same week (Fig. 1).

Super lure traps. Trap capture in 10 mg (super lure) baited traps was directly proportional to treatment rate, and the relationship was significant ($F = 12.06$, $df = 4$, $P < 0.0001$) (Fig. 2). Traps in blocks with membrane dispensers caught the least amount of males with a mean of 0.006 ± 0.011 *P. robiniae* per trap per week, followed by the traps in blocks treated with 7.41g/ha of pheromone that had a mean of 1.74 ± 0.61 *P. robiniae* males per trap per week. Traps in blocks treated with 2.47g and 1.24g/ha of pheromone

had very similar capture results with means of 2.91 ± 0.61 and 3.02 ± 0.87 *P. robiniae* males per trap per week, respectively. The 10 mg traps in control blocks caught the most moths with a mean of 7.02 ± 0.88 *P. robiniae* males per trap per week. We found that location (where the 10 mg traps were placed within a unit, i.e., NW, C, SE) had no significant effect on trap capture ($F = 1.14$, $df = 2$, $P = 0.3206$) and there were no location by treatment effects ($F = 0.31$, $df = 8$, $P = 0.9632$). Time did have a significant ($F = 4.66$, $df = 18$ $P < 0.0001$) effect on trap capture. Over the 18 weeks, trap capture decreased equally in all treatments. We found no significant time by treatment effects ($F = 0.99$, $df = 68$, $P = 0.4932$), time by location effects ($F = 0.29$, $df = 34$, $P = 1.0000$), or time by location by treatment effects ($F = 0.18$, $df = 136$, $P = 1.0000$).

When entire plantations are saturated with *P. robiniae* sex pheromone the 10 mg trap capture provided more resolution of *P. robiniae* activity than 1 mg trap capture. Our results from Potlatch's plantation in 2004 show that there are significant time by lure (10 mg or 1 mg) interaction effects on trap capture ($F = 3.06$, $df = 27$, $P < 0.0001$). At the peak of the flight period, we found that 10 mg traps caught significantly more male *P. robiniae* than 1 mg baited traps (Fig. 3).

Pheromone Formulations. Flowable. We found a significant difference in the number of *P. robiniae* captured over time ($F = 6.00$, $df = 8$, $P = 0.0002$) in traps baited with 25 mg of the flowable pheromone loaded onto filter paper. The mean trap catch the week after placement was 292.5 ± 267.54 *P. robiniae* males per trap and after four weeks the traps were still catching a mean of 202.75 ± 177.94 *P. robiniae* males per trap. The capture rate then dropped to a mean of just 14.5 ± 4.67 *P. robiniae* males per trap the fifth week and remained low for the rest of the experiment.

Membrane Formulation. Pheromone release from the membrane dispensers loaded with 24 mg of pheromone remained adequate from 1 June until the end of the *P. robiniae* flight period. After one month in the field, membranes sampled still had a mean of 16.37 ± 0.72 mg of active ingredient. The release of pheromone remained constant with a mean of 2.14 ± 1.31 mg remaining in the dispensers sampled 6 October 2003.

Damage Assessment. *Damage Surveys.* There were no significant differences in damage to sentinel trees between any treatment rate applied after 1 June 2003 ($F = 1.71$, $df = 4$, $P = 0.17$). Experimental blocks at Potlatch had significantly more damage in first- and second-year trees than any other farm ($F = 5.78$, $df = 4$, $P = 0.009$), however there were no significant treatment rate by farm and age effects ($F = 1.64$, $df = 11$, $P = 0.1238$). Since delayed application of pheromone in 2003 contributed to the lack of significant differences in damage related to rates used, we pooled the damage results for all rates including controls, and used that damage estimate to compare damage in subsequent years 2004 and 2005. Furthermore, because there were differences in amount of damage and application strategies (strip spraying versus border spraying) in 2005, farms were analyzed separately after 2003.

Survey results from Potlatch showed a significant reduction in damage from 2003 to 2005 ($F = 8.45$, $df = 2$, $P = 0.0005$) (Fig. 4). In 2003, we found a mean of 0.78 ± 0.12 hits per sentinel tree regardless of rate of pheromone used. The amount of damage was significantly ($P = 0.0232$) reduced in 2004 to a mean of 0.40 ± 0.11 hits per tree. A mean of 0.09 ± 0.12 hits per tree was found in 2005 after strip spraying, which was significantly ($P = 0.001$) less than damage found 2003 but not significant ($P = 0.0612$) from damage found in 2004. At Potlatch, from 2003 to 2005, we found no significant

differences in amount of damage in the two age classes we surveyed (first compared to second-year trees) ($F = 1.04$, $df = 1$, $P = 0.3122$). We also found no significant age (first- and second-year trees) by year (2003, 2004, and 2005) effects at Potlatch ($F = 0.91$, $df = 2$, $P = 0.4085$).

Survey results from the Sand Lake farm showed no significant differences in damage to first- and second-year trees from 2003 to 2005 ($F = 2.46$, $df = 2$, $P = 0.1017$) (Fig. 5). However, from 2003 to 2004 we did see a marginally insignificant ($F = 3.11$, $df = 2$, $P = 0.0576$) reduction in damage from a mean of 0.28 ± 0.08 hits per tree in 2003 to a mean of 0.089 ± 0.02 hits per tree in 2004 (Fig. 5). We saw the amount of damage then increase in 2005 after border spraying, back to a mean of 0.21 ± 0.07 hits per tree. We found no significant age effects between first- and second-year trees at Sand Lake ($F = 0.25$, $df = 1$, $P = 0.6236$) and no age of tree by year interaction effects ($F = 0.70$, $df = 1$, $P = 0.4104$).

Location of Damage. Over all the farms, we found significant differences in the location of damage occurrence in first-year trees ($F = 16.94$, $df = 1$, $P < 0.0001$). We also found a significant interaction effect between the location of damage on first-year trees and year that they were planted ($F = 4.34$, $df = 2$, $P = 0.0160$) (Fig. 6). Damage occurring at the base of the tree (0.29 ± 0.02 hits per tree) was significantly greater than damage occurring above the base of the tree (0.13 ± 0.02) when pheromone application was delayed in 2003 ($P < 0.0001$). Damage at the base of the tree was significantly reduced to 0.12 ± 0.02 hits per tree ($P < 0.0001$) in 2004 and to 0.09 ± 0.04 hits per tree in 2005 when pheromone application preceded first moth flight. Damage above the base of the

tree was slightly reduced in 2004 to a mean of 0.09 ± 0.02 hits per tree and significantly reduced to a mean of just 0.03 ± 0.03 hits per tree in 2005 ($P = 0.011$).

Replant Data. Potlatch's replant data showed significant interaction effects between year and need to replant in regard to percentage of sticks replanted ($F = 4.94$, $df = 8$, $P < 0.0001$) (Fig. 7). Over five years (2001 to 2005), we found no differences in the mean percent of first replant needed (2.51 ± 0.89 , 4.14 ± 1.24 , 2.54 ± 0.51 , 3.76 ± 0.97 , 3.78 ± 1.53 percent replanted, respectively). The need for second replant efforts steadily increased from a mean percent of 0.00 percent replanted in 2001 to a maximum of 2.96 ± 1.28 percent replanted in 2003. The need for a third replanting increased from 0.00 percent in 2001 to a maximum of 7.44 ± 2.44 percent replanted in 2003. There was no need for third replants in 2004 or 2005 (Fig. 7).

The non-linear regression from the head-capsule-width data of larvae removed from lodged trees was significant ($F = 111.1080$, $df = 1$, $P = 0.0005$), showing a consistent increase in larval head capsule width during six weeks in mid-season. These results suggest that eclosion from eggs occurred on or before approximately 7 June 2003 (Fig. 8).

Discussion

Pilot Experiment. The full rate in our pilot test, one septum loaded with 1 mg placed on every tree (400 trees), with trees spaced for pulp (1,285 trees per ha), was roughly 1.29 g of *P. robiniae* sex pheromone per ha. However, these septa were used for only seven weeks in 2002, and we did not know how the release rate of septa would compare to formulations (flowable or membranes) to be used in 2003. We hypothesized that our medium dosage for 2003 should be 2.47 g of *P. robiniae* sex pheromone applied

to a hectare of trees per season and that this amount should be more than adequate to confuse the male moths so that they could not find mates.

Trap Shutdown 2003. *Monitoring traps.* Trap monitoring in 2003 showed almost complete shutdown of 1 mg baited traps within seven days of the first pheromone application. These results also showed that we had selected areas with high population pressure of *P. robiniae* for pheromone applications (Fig. 1). Had conventional insecticides been used or had nothing been done in 2003, the number of male moths captured for that season would likely have exceeded the outbreak year of 2002 (Brown et al., 2006). Therefore, we did not avoid pest pressure in selecting sites for pheromone treatment in 2003; to the contrary, we actually targeted units with the highest pest pressure, with the least amount of pheromone tested (1.24 g/ha), and still we were able to shut down trap capture of male *P. robiniae*. Starting pheromone control strategies in areas with high populations have not succeeded in codling moth control without insecticide support (Brunner et al., 2002). Insecticide treatments have been shown to be inadequate for *P. robiniae* control (Brown et al., 2006) and the units we targeted were selected based upon value to the companies.

Super lure traps. Results from the super lure (10 mg) baited traps, showed trap catches were inversely proportional to the rates of pheromones used in 2003 (Fig. 2). The variation between trap catches showed that different pheromone rates affected the number of male moths caught per trap. As with the 1 mg baited traps, super lure trap catches did not show whether pheromone control was effective in preventing mating of females.

The purpose of the super lure (10 mg) baited traps (Gut and Brunner, 1996; Knight and Light, 2005; Knight et al., 2005) was to put out a greater concentration of pheromone, thereby making that trap more apparent than 1 mg baited traps to male *P. robiniae*. We placed three super lure (10 mg) traps in each experimental block in 2003. Trap catches from the three locations did not differ significantly, therefore in 2004 and 2005 we eliminated the NW and SE traps and left only the centrally located super lure monitoring traps. Our comparison of capture rates in 2004 of 1 mg to 10 mg baited traps showed that the super lures did just what they were supposed to do. At the peak of the flight period 10 mg baited traps caught significantly more male *P. robiniae* than 1 mg baited traps. With almost complete shutdown of 1 mg baited traps, the added resolution of *P. robiniae* flight activity provided by the super lure traps was important. The pheromone-based control strategy depended on monthly pheromone applications lasting 30 days. An increase in trap capture may indicate declining pheromone concentration in the field. While 1 mg traps may not detect an increase in male *P. robiniae* capture, the increased attractiveness of the super lure (10 mg) traps should be more sensitive, thereby alerting IPM managers that another application of pheromone is needed.

Pheromone Formulations. *Flowable.* The result of our flowable pheromone longevity test validated the manufacturer's recommendations. We found that 25 mg of pheromone loaded onto filter paper lost considerable effectiveness after four weeks. These results corroborate our recommendations to pest managers that flowable pheromone applications be made every 30 days or when they begin to record increases in the number of *P. robiniae* captured in monitoring traps.

Membrane Formulation. Twenty four (24) mg of pheromone loaded into the membrane dispensers in 2003 proved to be an adequate amount to release an effective concentration of pheromone over four months. The increased pheromone load from 24 mg to 40 mg was recommended to extend the release through an extra two months (from April through September 2004) and to ensure that only one application of membranes would be needed.

Comparisons of membrane and flowable pheromone formulations have been made for *Grapholita molesta* (Busck) and *Cydia pomonella* (L.). Trimble et al. (2004) found that significantly fewer *G. molesta* were caught in areas treated with a hand-applied pheromone dispenser than with flowable pheromone applied with an air-blast sprayer. However, Knight and Larson (2004) found that the location of the flowable pheromone application in relation to the canopy affected males' ability to locate virgin female baited traps. There were significantly fewer virgin female baited traps catching male codling moths when the flowable pheromone was applied to the top of the canopy than when the pheromone was applied with an air-blast sprayer (Knight and Larson, 2004). We also found that in our units treated with hand-applied membrane dispensers, we caught significantly fewer *P. robiniae* in monitoring traps. At the same time, we found no difference in the amount of damage when membrane dispensers were compared with flowable pheromone treatments in 2003. The flowable pheromone applications in hybrid poplars are being made by aircraft directly to the top of the canopy, in a low volume of water, which according to Knight and Larson (2004) is more effective than pheromone application made with high volume air-blast sprayers. For economic reasons,

membrane dispensers were not used in hybrid poplars in 2005, yet less damage was reported than in 2004 even though only flowable pheromones were in use.

Damage Assessment. *Damage Surveys.* Although trap shutdown was impressive, it did not demonstrate control. Trap shutdown only shows that in the presence of pheromone, males cannot find the pheromone-baited trap; it does not ensure that males have not found and mated with females. Several researchers (Cameron et al., 1974; DeBarr et al., 1983; Daterman et al., 1985; and Atanassov et al., 2002) have reported success in their efforts to confuse male moths, but failed to reduce pest populations and/or damage to the crop.

There are three main ways to show the effectiveness of a pheromone control strategy: show that females have not been mated, show a reduction in immature populations, or show a reduction in damage to the host plant (Cardé and Minks, 1995). We have not been able to effectively capture the necessary numbers of diurnally active females to determine if they are mated or not. In fact, after three years of pheromone use, it is difficult to locate any *P. robiniae* in the poplar plantations. In order to show a reduction of immature populations, we would have to destructively sample poplar trees; this is not feasible because the trees are the product in this system. This leaves us with reduction in damage as the only way to demonstrate the effectiveness of the pheromone control strategy.

We have demonstrated successful pheromone control by reducing of damage due to *P. robiniae* in 2004 and 2005 (Fig. 4 and 7). We started the pheromone control strategy in 2003, but because of problems in acquiring adequate amounts of pheromone and getting the proper authorization to use an unregistered product over large acreage,

implementation was delayed until 1 June 2003, about a month and a half after the beginning of *P. robiniae* flight period. Pheromone-baited traps were immediately shut down, but when the different treatment rates were compared, there was no significant difference in damage to first- and second-year trees in 2003. We believe this can be directly attributed to the late first application of pheromone and the fact we only had enough pheromone to target 2,948ha (only first- and second-year trees).

In 2004, we documented significantly less damage to first- and second-year trees at Potlatch's plantation than in 2003 (Fig. 4). Although not significant, we also recorded less damage in 2004 at Sand Lake (Fig. 5). We believe the success in 2004 was the direct result of a timely first application of pheromone (1 April) and complete pheromone saturation of entire farms.

We recorded another reduction in damage to first- and second-year trees at Potlatch's plantation in 2005. This reduction was not significantly less than damage recorded in 2004; however, it was a (> 50%) reduction in mean *P. robiniae* hits per tree. At the Sand Lake farm in 2005 we saw a resurgence of damage to first- and second-year trees. We hypothesize that this renewed damage at Sand Lake was due to an altered pheromone application strategy used on this farm. For economic reasons, GreenWood Resources decided to treat only first- and second-year trees plus a buffer spray zone surrounding these units. This was the basic strategy used in 2003 when the supply of pheromone was limited. This "island" strategy allows for mated females to emigrate from untreated areas and oviposit in treated areas; according to Cardé and Minks (1995), "The most crucial trait in determining a pest susceptibility to synthetic pheromone mating disruption is the prevalence of migration by mated females."

Damage at Potlatch in 2003 was significantly greater than in any other farm studied. Sand Lake has always had a smaller population of *P. robiniae* than Potlatch (Fig. 4 & 5). Different pheromone application strategies used by the different companies after 2003 prohibited us from making direct comparisons between the different farms. However, at Potlatch we saw a consistent decrease in damage from 2003 to 2005, which we attribute to their continued treatment of nearly all acreage. Potlatch, for economic reasons, implemented strip spraying in 2005, a strategy that relied on pheromone drift to cover two thirds of the trees older than three years. However, this strategy still provided adequate coverage, because with each pheromone application the strips were rotated so that by the end of the six monthly applications, every strip within the entire unit had been treated with pheromone twice.

Location of Damage. We recorded the majority (~66%) of the damage to first- and second-year trees in 2003 at the base of the tree (within 6 cm of the ground) (Fig. 6), which is the portion of new growth exposed to moths before late pheromone applications started on 1 June. The head-capsule-width data of larvae removed from lodged trees in 2003 suggests that egg hatch occurred before 158 days on the Julian calendar (7 June 2003) (Fig. 8). From our unpublished data we know that it takes about 14 days from oviposition to egg eclosion at 21°C, which suggests that these larvae sampled (n =119) from lodged trees in 2003 were from eggs oviposited prior to the first pheromone application. There was a significant reduction in base (< 6cm) hits in 2004, which suggests that early application of pheromone reduced the infestation rate in first-year trees. This also supports our hypothesis that the majority of damage that occurred in 2003 was from the progeny of females mated prior to the 1 June first application of

pheromone. In 2005, we documented a slight reduction in damage at the base of the tree, but more importantly we documented a significant reduction in damage occurring above the base of the tree, which suggests a reduction in the population of *P. robiniae* attacking trees later in the growing season.

Replant Data. Replanting information provided us with an immediate evaluation of the pheromone control strategy. Planting and replanting of harvest fields occurs in the second quarter (April–June). Later replanting is essentially impossible, as the adjacent trees proceed to close the canopy at a uniform rate and any late-planted “sticks” are unlikely to survive. Initial planting occurs in late April and there are up to three consequent replanting of failed sticks. Every stick planted and/or replanted was documented. First replants are attributed to inviable sticks. Second and third replantings, however, have historically been attributed to *P. robiniae* infestation, easily documented by lodged saplings infested with *P. robiniae* larvae. Replanting records show a steady increase in the need for second and third replantings from 2001 to 2003 (Fig. 7). We saw the largest number of failures (43,242) resulting in the need for a third replanting in 2003, suggesting a peak population of *P. robiniae*. After two years of pheromone applications, there has been no need for a third replanting and the second replanting has been significantly reduced to levels comparable to pre-2001, before *P. robiniae* was a problem.

Conclusions. In summary, conventional insecticides were not efficacious against the outbreak of western poplar clearwing moths seen in 2002 (Brown et al., 2006). The pheromone-based strategy initiated in 2003 was flawed due to delayed application and insufficient availability of pheromone, the combination of which resulted in failure to treat overwintering *P. robiniae* before they were mated and laid eggs. Complete

saturation of poplar plantations with *P. robiniae* pheromone in 2004 significantly reduced damage to sentinel trees and eliminated the need for second and third replanting efforts. Strip spraying, relying on pheromone residue and drift to cover untreated areas, may provide some economic relief for growers. Border sprays surrounding treated areas seem to offer no extra protection against *P. robiniae* females emigrating from untreated areas to treated areas. A long-term strategy for insect pest management in poplar will depend upon clone selection, but pheromone-based control can be a key interim component toward controlling *P. robiniae*.

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Tables:

Table 1. Pheromone applications to units in both Potlatch's and Boise's plantations in 2003. The numbers of irrigation units treated or not treated are given within parentheses, followed by the area treated or not treated in hectares.

Formulation	Boise	Potlatch	Total
Membranes (6.2 g/ha/season)	(3) 180	(3) 182	(6) 362
Flowable (7.41 g/ha/season)	(3) 182	(3) 184	(6) 366
Flowable (2.47 g/ha/season)	(11) 669	(8) 505	(19) 1,174
Flowable (1.24 g/ha/season)	(12) 857	(3) 189	(15) 1,046
Controls	(6) 432	(8) 503	(14) 935
Total area within test	(35) 2,320	(25) 1,563	(60) 3,883
Total area not treated	(65) 3,961	(67) 5,147	(132) 9,108
Total hectares	(100) 6,281	(92) 6,710	(192) 12,991

Figures:

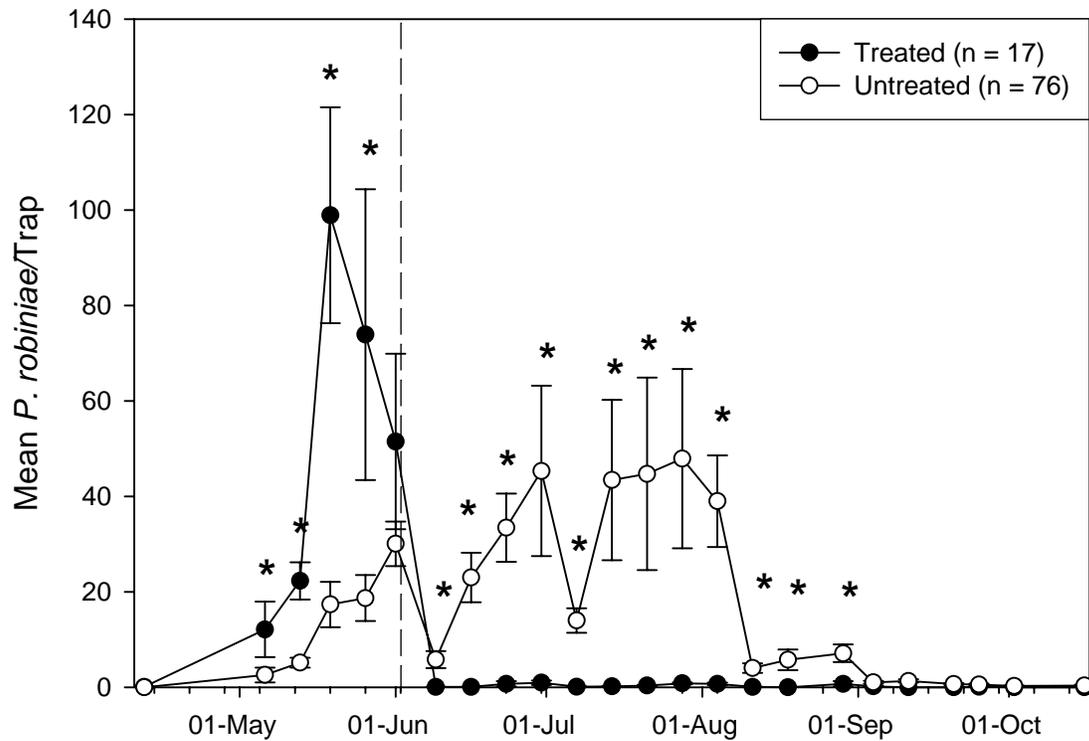


Figure 1: Male *P. robiniae* capture in 1 mg baited monitoring traps from Potlatch’s plantation in 2003. Vertical dotted line represents the start of the pheromone control strategy, thus “treated” units were not actually treated until 1 June. An asterisk (*) represents a significant difference ($P < 0.05$) between the two treatments for that week.

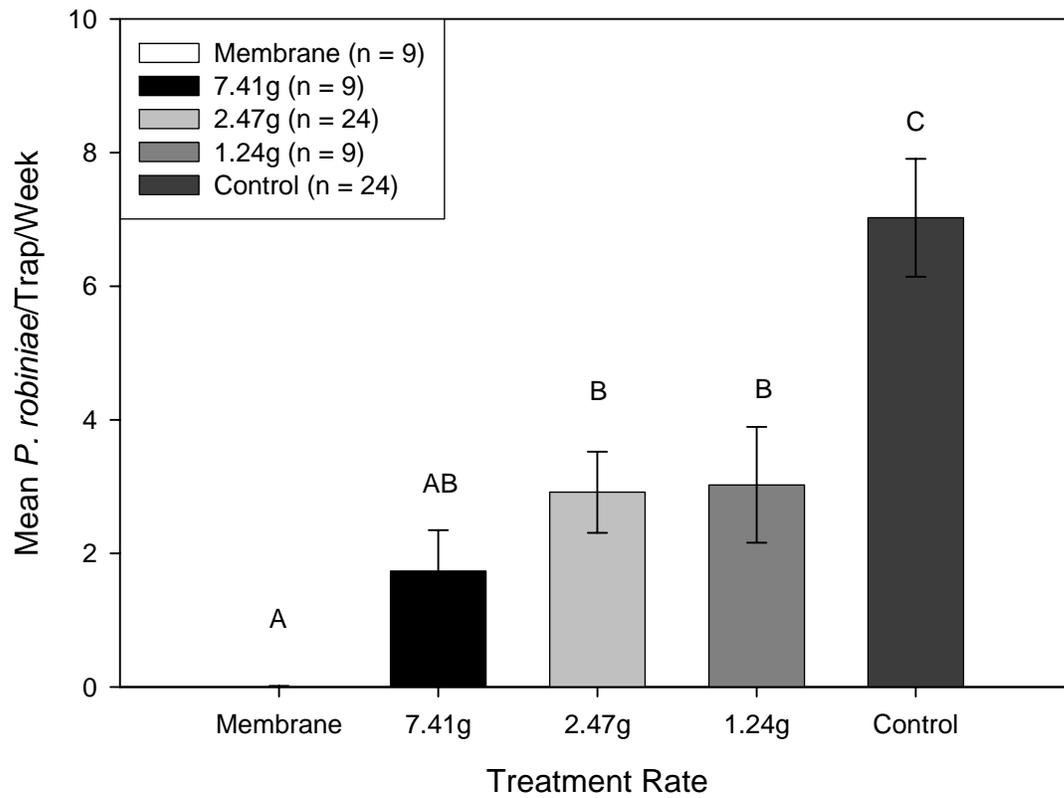


Figure 2: Male *P. robiniae* captured in 10 mg (super lure) baited traps. Flowable concentrations (1.24g to 7.41g/ha) are total season-long amounts of pheromone applied, whereas membranes loaded with 24 mg of pheromone were applied once. Means with the same letter are not significantly different at $P < 0.05$.

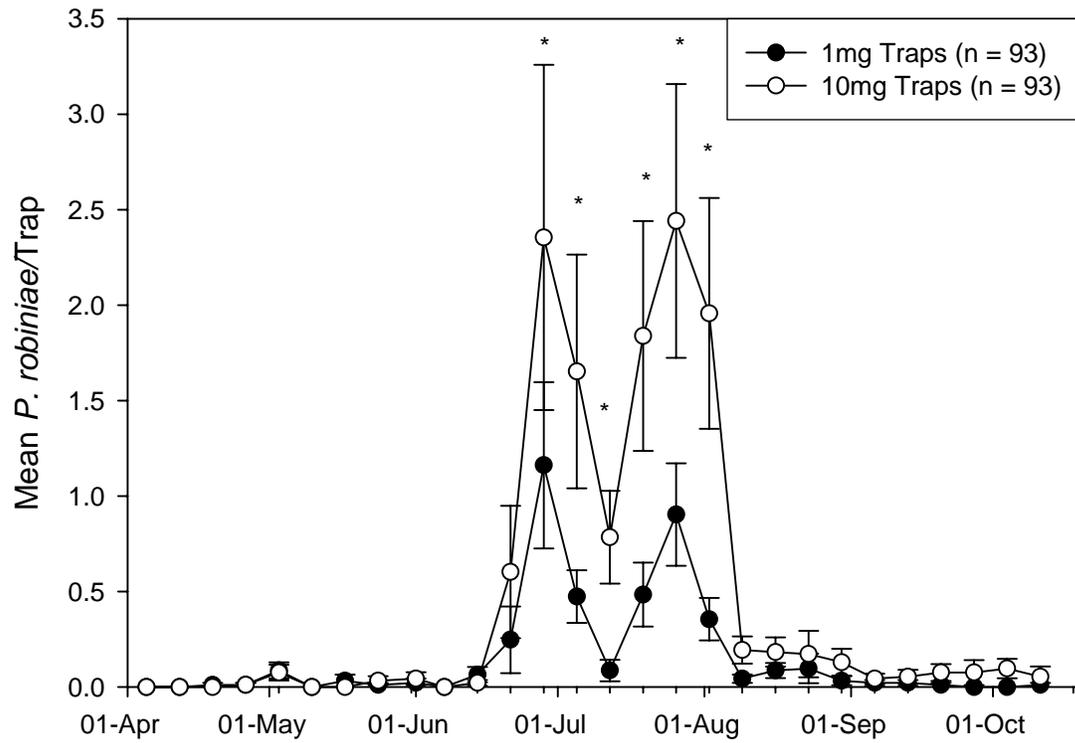


Figure 3: Male *P. robiniae* captured in 2004 at Potlatch showing the differences between 1 mg and 10 mg baited traps. Asterisks (*) denote a significant difference between trap capture for the given date ($P < 0.05$).

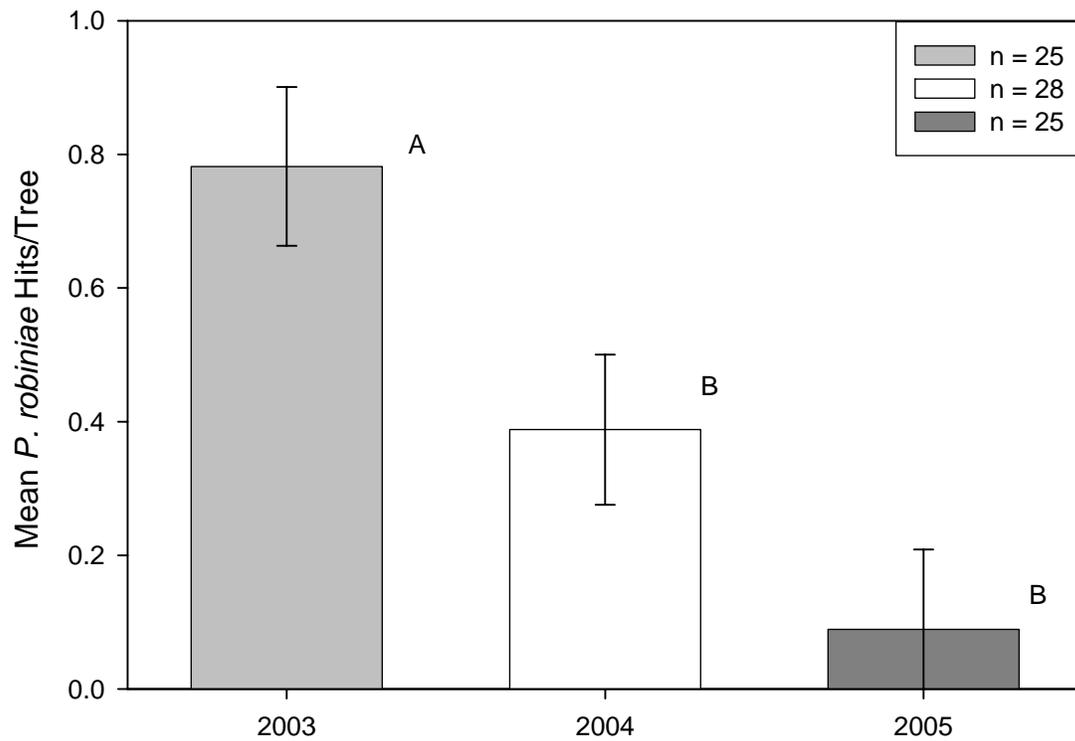


Figure 4: Damage survey results for three years (2003-2005) from only Potlatch's plantation. N = the number of units involved and 30 trees were surveyed in each unit. Means with the same letters are not significantly different at $P < 0.05$.

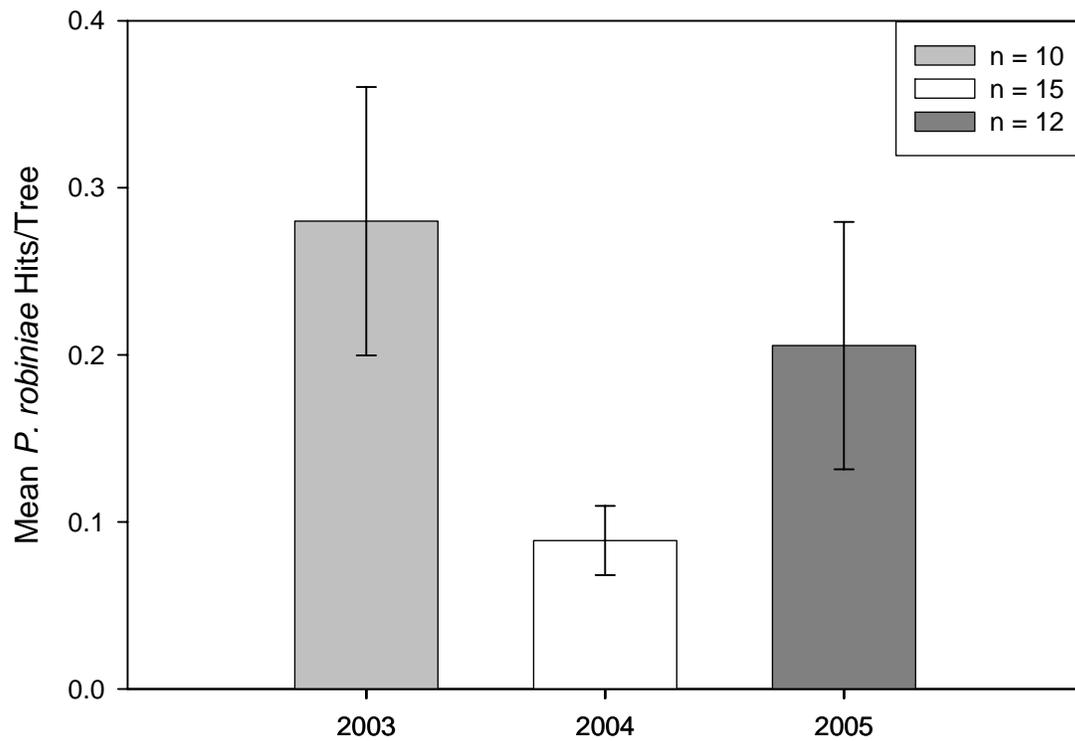


Figure 5: Damage survey results from only the Sand Lake farm for three years (2003-2005). N = the number of units involved and 30 trees were surveyed from each unit. There were no significant differences between years ($P < 0.05$).

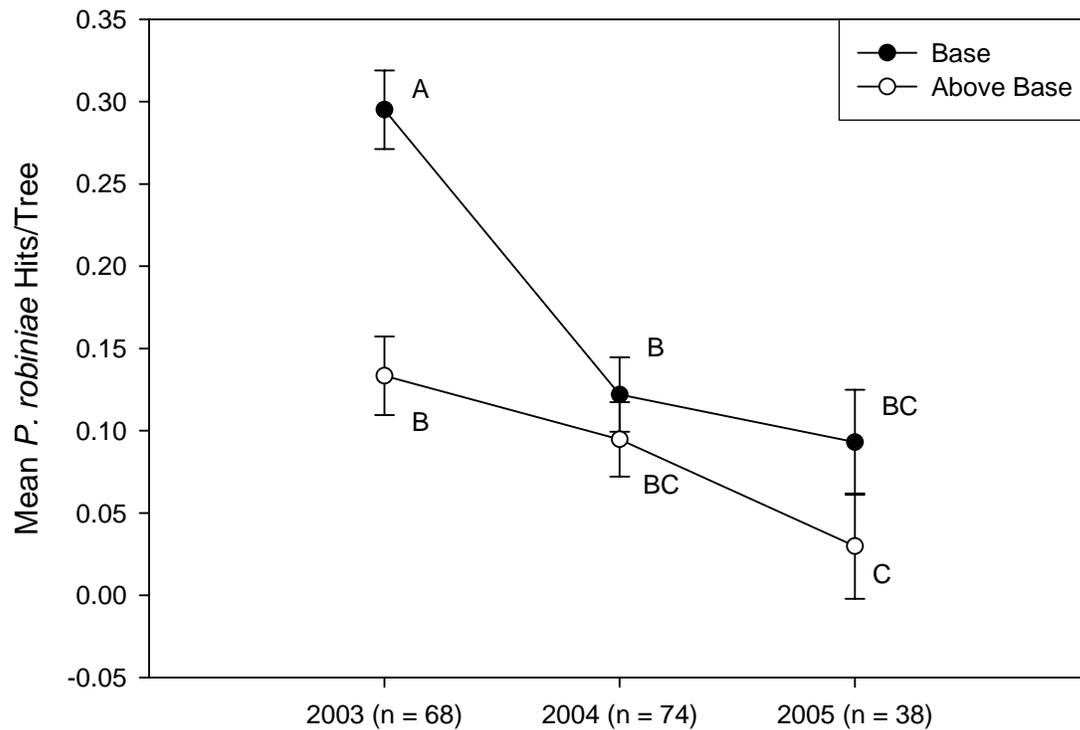


Figure 6: Location of damage occurrence. “Base” is within 6cm of the ground and “Above Base” represents the whole tree above 6cm from the ground. N = the number of units involved; 30 trees were sampled from each unit. Means with the same letters are not significantly different at $P < 0.05$.

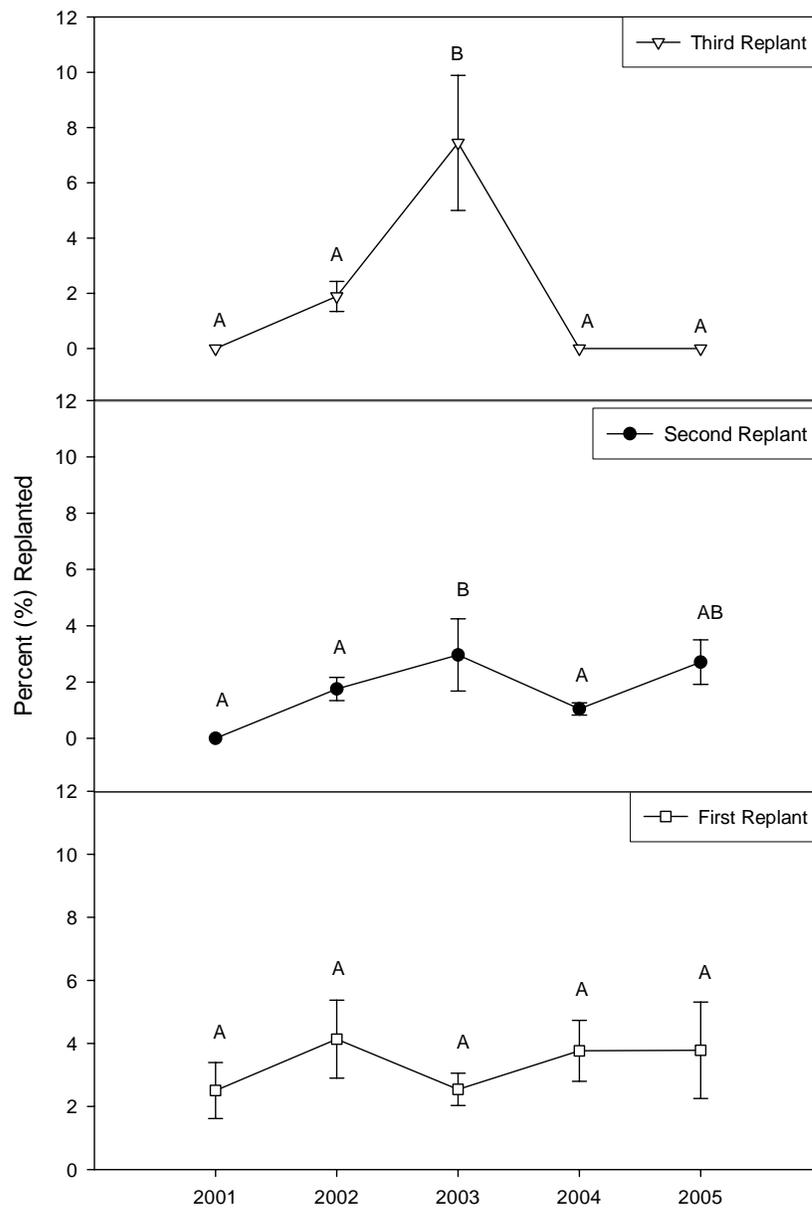


Figure 7: Replant data from Potlatch’s plantation for five years (2001-2005). First replant is attributed to stick inviability. Second and third replants were attributed to *P. robiniae* damage, except in 2005 where second replant was attributed to an unknown soil arthropod. Means for each replant with the same letter are not significantly different at $P < 0.05$.

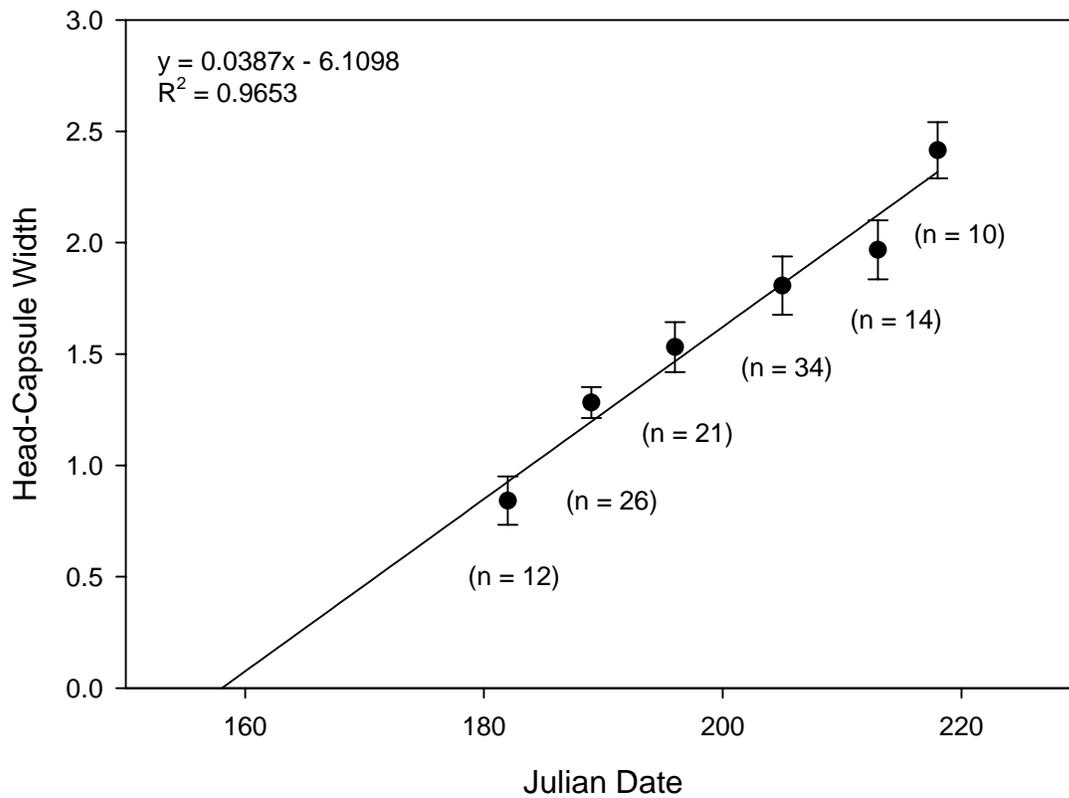


Figure 8: Head-capsule widths of larvae removed from newly planted lodged trees between 1 July and 6 August 2003 set to a Julian calendar. N = number of larvae sampled. Regression line shows approximate date of neonate hatch (158 days, 7 June 2003). Linear regression is significant at $F = 111.11$, $df = 1$, $P = 0.0005$.

Chapter 5: Pheromone drift and a canary species.

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ABSTRACT: A pheromone based control strategy was initiated toward controlling the western poplar clearwing moth (*Paranthrene robiniae* Hy. Edwards) in hybrid poplar plantations in 2003. There was not enough pheromone to treat all poplar plantings, so priority was given to younger trees, resulting in a checkerboard pattern of units treated among untreated units. Immediately it was clear that pheromone applications influenced trap capture of moths in units adjacent to or downwind of the actual application site. After saturating the poplar plantations with pheromone in 2004, populations of *P. robiniae* decreased dramatically and pest managers interested in reducing their cost instituted a “strip spraying” or border application methodology. With strip spraying, only one-third of each unit was treated each month, thus relying upon drift of the applied pheromone’s plume to suppress mating of *P. robiniae* in units adjacent to or downwind of the point of application.

Once the pheromone based control strategy was initiated in the hybrid poplar plantations, it became difficult to monitor *P. robiniae* populations and in situ pheromone concentrations between applications. Normal monitoring traps were completely shut down after each pheromone application. Repeated seasons of pheromone use suppressed *P. robiniae* populations to the point that even the 10 mg lures were no longer reliable indicators that the pheromone concentration had diminished to a point that allowed males to locate the traps.

An innocuous moth, *Nemapogon variatella* (Clemens) was attracted in great numbers to traps baited with *P. robiniae* sex pheromone. When *P. robiniae* pheromone was applied to a unit of poplar trees, both *P. robiniae* and *N. variatella* males were no longer captured in *P. robiniae* sex pheromone baited traps. In the absence of *P. robiniae*

males, *N. variatella* males can be used to monitor *P. robiniae* sex pheromone concentrations in the field. *Nemapogon variatella* has become the “canary” species for *P. robiniae* sex pheromone concentration; when trap capture of *N. variatella* males increases, it is time to apply more pheromone. We have no evidence that *N. variatella* populations have declined after repeated years of *P. robiniae* sex pheromone applications.

KEYWORDS: pheromone monitoring, varying concentrations, indicator species, mating disruption

Introduction

Timber producing corporations, Potlatch (Boardman, OR), Boise (Wallula, WA), and GreenWood Resources (Portland, OR) have established plantings of hybrid poplar on approximately 14,164 ha (35,000 acres) in eastern Oregon and Washington. Most of these trees are harvested for pulp, but because of declining pulp prices, one company (Potlatch) has made a market-based decision (Stanton et al. 2002) to convert its management practices toward the production of non-structural sawtimber.

As larvae, western poplar clearwing moths *Paranthrene robiniae* (Hy. Edwards) (Lepidoptera: Sesiidae) damage trees by burrowing into the bole or limbs of trees, compromising the structural integrity such that moderate to high winds can cause limbs and trunks to break. Besides damage to mature trees, newly planted saplings infested by *P. robiniae* easily lodge.

A pheromone control strategy has been implemented to manage the damage caused by *P. robiniae*. Kittelson et al. (unpublished) have documented a reduction of damage caused by *P. robiniae* after farms were saturated with synthetic sex pheromone. With the decreasing population of *P. robiniae*, farm managers have begun experimenting with ways to reduce the economic burden of saturating entire farms with the synthetic *P. robiniae* sex pheromone. One company is using a “strip spraying” method, where only one third of each unit is being treated at a given time and relying on drift to cover the untreated portions. Another company is only applying pheromone to specific high value units plus a buffer surrounding them.

We noticed in 2003, when our pheromone control strategy only targeted first and second yr trees, that pheromone applications seemed to affect trap catches of male *P.*

robiniae in units that were not treated with pheromone. With pheromone applications made to individual units in a “checkerboard” pattern and without complete saturation in 2003, we were able to make direct comparisons between treated and untreated units in relation to spatial arrangement and predominant wind direction. We also setup an experiment with multiple pheromone baited traps (one trap/0.4 ha) to determine the distribution of *P. robiniae* within a block (16 ha) of poplars.

With declining populations of *P. robiniae* and less pheromone being applied, monitoring the effectiveness and longevity of pheromone applications is important, yet difficult to assess. We have found that a moth, *Nemapogon variatella* (Clemens) is attracted to *P. robiniae* sex pheromone, in large quantities. By monitoring the catch of *N. variatella* in traps baited with *P. robiniae* sex pheromone, relative ambient pheromone levels maybe assessed without male *P. robiniae* present. There are many examples of successful pheromone control strategies in the literature (Carde and Minks 1995) and a few examples of a pheromone being used to control multiple species (Ridgeway et al. 1990, Carde and Minks 1995, Evenden et al. 1999, Judd and Gardiner 2004) but to our knowledge there are no examples of a non-pest species being used as an indicator species for monitoring the concentrations a sex pheromone for the pest species.

Materials and Methods

Terminology. Several different terms will be used throughout this manuscript to describe an area of poplar trees. A plantation is the entire holding of one company, a farm is the largest contiguous subunit of a plantation, irrigation unit will describe a managed portion of a farm irrigated by a common manifold station, a planting unit is a

smaller portion of an irrigation unit, and the term block will be reserved for our experimental areas.

Ages of the units will be referred to by the upcoming growing season and not actual age of trees within said unit. So, a newly planted unit of trees will be referred to as first yr trees because they are in their first season of growth. Trees that are in their second season will be called second yr trees, third yr trees, etc.

Experiments were conducted beginning in 2002 through 2006 in intensively managed hybrid poplar farms in northeastern Oregon and southeastern Washington. These areas were high desert scrub that have been converted to irrigated agriculture, with water pumped from the Columbia and Snake rivers.

Monitoring Traps. All monitoring traps were Unitrap® bucket traps baited with 1 mg of *P. robiniae* sex pheromone purchased from Suterra LLC (213 SW Columbia, Bend, OR), unless otherwise specified. The synthesized pheromone blend consisted of a 4:1 ratio of (E, Z):(Z, Z)-3,13-octadecadienyl straight-chain 18 carbon alcohols (ODDOH) (Cowles et al. 1996). Each trap had a ~2 cm² piece of Vapona® (dichlorvos) No-Pest Strip (Monsanto Canada Inc, Mississauga, Ontario) to kill the trapped moths. One trap baited with 10 mg of pheromone, referred to as a “super” lure, was placed in each unit as well. All bucket traps were thoroughly cleaned before deployment. Each trap was monitored weekly. The number of male *P. robiniae* were recorded, and the traps were emptied. Lures and killing strips were replaced every other month.

***P. robiniae* Spatial Distribution.** Forty bucket traps baited with 1 mg lures were placed in a 16 ha block of trees in a grid spacing of one trap every 0.4 ha. Traps contents

were counted, recorded and emptied weekly for 12 wks. We had three replications of 16 ha blocks in independent irrigation units.

Pheromone Drift. *Adjacent blocks.* In 2003, at Potlatch's plantation, first and second yr trees were treated with *P. robiniae* sex pheromone in an attempt to control mating. Throughout the growing season 93 traps baited with 1 mg lures were monitored in treated and untreated units. Potlatch's plantation consists of square or rectangular units of either 65 or 110 ha each. Treated units were interspersed with untreated units in such a way that they shared either borders or corners in a checkerboard design. Untreated units in the South farm at Potlatch were split into four categories based on how many treated units they bordered. Units that bordered no treated units were designated "Zero" (0), untreated units that had one treated unit sharing either a border or a corner were labeled "One" (1). "Two" (2) designated untreated units sharing borders or corners with two treated units, and "Three" (3) shared borders and/or corners with three treated units.

Downwind Traps. Boise's plantation consists of units of variable sizes (28.7 to 176.5 ha) and shapes; therefore borders and corners were irregular. At Boise, in 2003, we followed the same approach as at Potlatch with monitoring traps in treated and untreated units, but instead of classifying them based on bordering units we classified traps in untreated units based upon the predominant wind direction in respect to treated units. Due to insufficient number of traps in all directions only traps that we could classify as being directly upwind or directly downwind were used in the analysis. The predominant winds are from the southwest (225°) toward the northeast (45°). Traps in untreated units located 210° to 240° toward the southwest of treated units were

considered upwind, and traps in untreated units located 30° to 60° to the northeast of treated units were considered downwind traps.

Farms isolated from pheromone. No *P. robiniae* pheromone applications were made on Potlatch's North farm or at Boise's Wallula farm in 2003. We analyzed monitoring traps on these farms for any influence of pheromone drift. Potlatch's North farm consists of 29 units (3,025 ha) located 0.8 to 5.6 km downwind from the nearest pheromone treated units. Boise's Wallula farm had 6 units (446 ha) that were at least 7.4 km upwind and 56 km downwind from any pheromone treated units.

Nemapogon variatella. For identification purposes, live *Nemapogon variatella* adults were captured flying around bucket traps baited with a 1 mg *P. robiniae* sex pheromone lure. Specimens were pinned and submitted to Jean-François Landry at the Canadian National Collection of Insects, Ottawa, Ontario for identification.

Trap catches of *N. variatella* tended to be quite large. When trap catches of *N. variatella* were less than 100/trap, individuals were counted. For large quantities of *N. variatella*, volume estimates were recorded in the field. Volumes of moths in graduated cylinders were converted to an estimated number of moths using 120.8 ± 4.4 *N. variatella* per ml.

Effects of Pheromone on N. variatella. In 2003, monitoring of *N. variatella* caught in traps baited with *P. robiniae* sex pheromone began 16 June, and were made weekly along with the *P. robiniae* counts. Male *P. robiniae* were counted and removed from the traps and then the volume of *N. variatella* males was measured using graduated cylinders.

Varying Concentrations of Paranthrene robiniae pheromone. Concentrations of Checkmate WPCM-flowable (EPA Reg. No. 56336-40, Suterra LCC, Bend OR) containing 0.01, 0.1, 0.5, and 1.0 mg of *P. robiniae* pheromone per ml were used to attract *N. variatella*. One ml of each concentration was pipetted onto individual 55 mm filter papers (Whatman International LTD, Maidstone, England) that were placed into bucket traps. A commercial septum (Suterra LCC) loaded with 1 mg of *P. robiniae* sex pheromone and filter paper with only water served as upper and lower limit controls, respectively. These six bucket traps baited with the varying concentrations of *P. robiniae* pheromone were positioned in an untreated unit, the trap with the septum was positioned furthest downwind, the highest concentration of Checkmate[®] was positioned upwind of the septum, etc., with the water treatment placed the furthest upwind to minimize the influence of drift. Traps were left for one wk, after which the trap contents were counted and emptied. This setup was repeated 11 times in 11 independent units; at each location new filter papers were loaded with fresh pheromone.

N. variatella in strip sprayed units. Six bucket traps baited with 1 mg *P. robiniae* sex pheromone were placed inside strip sprayed units at Potlatch beginning 7 July 2006. The bucket traps were positioned in a transect running north and south and spaced 30 m apart. In these units, 30 m wide “strips” were sprayed running east and west, with 60 m of untreated area in between. This setup was replicated in three independent units. In addition, three untreated units were used as controls. In each control unit, two bucket traps baited with 1mg pheromone lures were positioned 30 m apart in the center of each 110 ha unit. Trap contents were counted and emptied weekly for six wks.

Statistical Analysis. Repeated measures ANOVA was used to analyze all experiments presented in this report except the varying *P. robiniae* pheromone concentration experiment for *N. variatella* (PROC GLM SAS, 2003). LSDs were used to identify differences between significant main effects and LSMEANs were used to identify differences in significant interaction effects (PROC GLM SAS, 2003). ANOVA using LSDs was used to analyze the difference in catches of *N. variatella* in traps with varying concentrations of *P. robiniae* pheromone. Data analyses for trap direction and traps with varying pheromone concentrations used LOG10 transformed to gain normal distribution. All graphical representations were made with SigmaPlot 9.01, Systat Software Inc. 2004.

Results

***P. robiniae* Spatial Distribution.** Our analysis of 40 traps positioned in a grid pattern with one trap every 0.4 ha showed a significant ($F = 2.65$, $df = 28$, $P < 0.0001$) row by trap interaction effect. The outside 22 border traps caught significantly ($F = 146.74$, $df = 1$, $P < 0.0001$) more *P. robiniae* males than the 18 traps positioned inside our experimental block (Fig. 1). We also found that there were significant time effects ($F = 5.62$, $df = 11$, $P < 0.0001$) over 12 wks, however we found no significant time by trap by row interaction effects ($F = 0.38$, $df = 308$, $P = 1.0000$), so we attribute the difference in trap counts over time to *P. robiniae* phenology.

Pheromone Drift. Adjacent Block. We found significantly ($F = 5.79$, $df = 4$, $P < 0.0001$) fewer male *P. robiniae* caught in baited traps in untreated units, if these units shared a border or corner with a treated unit, compared to trap catches in units completely surrounded by other untreated units (Fig. 2). Trap catches varied significantly from wk

to wk ($F = 1.70$, $df = 20$, $P = 0.028$), however, we found no significant ($F = 0.85$, $df = 80$, $P = 0.82$) wk by number of untreated units in contact with treated units interactions, so the we attribute the weekly variation in trap catch to phenology and not to an influence of the pheromone.

Downwind Traps. We recorded a significant ($F = 72.32$, $df = 1$, $P < 0.0001$) difference in trap catch of male *P. robiniae* based upon whether traps in untreated units were positioned directly up or downwind of treated units. Traps ($n = 5$) in untreated units, downwind of pheromone treated units caught a mean of 0.29 ± 0.11 *P. robiniae* per trap per wk, while at the same time traps ($n = 5$) directly upwind of pheromone treated units caught a mean of 45.24 ± 13.14 *P. robiniae* males per trap per wk (Fig. 3). We found no significant wk (time) effects ($F = 1.59$, $df = 17$, $P = 0.075$) and no significant wk by trap position (upwind or downwind) interactions ($F = 1.19$, $df = 17$, $P = 0.28$).

Farms isolated from pheromone. There were significant differences ($F = 8.45$, $df = 17$, $P < 0.0001$) in trap capture of *P. robiniae* over time (wk to wk) on Potlatch's North farm. We found a reduction in trap capture of *P. robiniae* males the wk following each pheromone application that was made on the South farm in 2003 (Fig. 4). Our analysis showed that only after the second and third pheromone application were the reductions significant, however after the first application the *P*-value from the LSMEANs was $P = 0.0746$, which is marginally significant. By the time the fourth pheromone application was made in September the population of *P. robiniae* had already begun to decline due to the phenology, however the reduction in trap catches was still noticeable.

In order to extend our analysis of the possible drift affect beyond our observations on the North farm, we included Boise's Wallula farm trap counts. The Wallula farm was

untreated in 2002 and 2003. Mean weekly trap catch did not differ significantly ($F = 1.03$, $df = 1$, $P = 0.3104$ between 2002 and 2003 and there were no significant ($F = 0.50$, $df = 16$, $P = 0.9445$) wk by yr interactions (Fig. 5). There were significant ($F = 2.44$, $df = 16$, $P = 0.0019$) differences from wk to wk but we attributed this to *P. robiniae* phenology and not to effects of distant pheromone applications.

Nemapogon variatella. All specimens were identified as male *Nemapogon variatella* [Lepidoptera: Tineidae]. Voucher specimens are located in the M. James Entomological Collection at Washington State University.

Effects of Pheromone on N. variatella. Results from our weekly monitoring traps in 2003, show *N. variatella* are highly affected, meaning males are attracted to traps baited with *P. robiniae* pheromone, and once an area is treated with pheromone, trap capture of *N. variatella* is shut down. Our data showed a significant difference ($F = 422.74$, $df = 1$, $P < 0.0001$) in trap capture of *N. variatella* moths based on whether the trap was positioned inside of a pheromone treated or untreated unit. We also found a significant interaction effect ($F = 2.95$, $df = 15$, $P = 0.0001$) between trap capture of moths and time in untreated units (Fig. 6). However, traps in units treated with *P. robiniae* pheromone never caught *N. variatella*, so the variation in untreated units over time can be attributed to *N. variatella* phenology. There is a possibility that drift from pheromone treatments affected the trap catch of *N. variatella* in untreated units, but from our abbreviated monitoring season of *N. variatella* beginning in June in 2003, we have no evidence of this.

Varying Concentrations of Paranthrene robiniae pheromone. Our results showed the number of *N. variatella* males trapped varied significantly ($F = 21.41$ $df = 10$, $P <$

.0001) when the lures being used to attract them had different pheromone concentrations (Fig. 7). The commercially bought septum containing 1 mg *P. robiniae* sex pheromone attracted the most, catching a mean of 632.65 ± 168.40 male *N. variatella* moths per trap. An equivalent amount (1 mg active ingredient) of flowable *P. robiniae* pheromone on filter paper caught only a mean of 183.85 ± 96.22 *N. variatella* males per trap. Trap catches of *N. variatella* males continued to decrease as concentrations of pheromone loaded on filter paper decreased, down to the 0.001 mg pheromone baited filter paper catching a mean of 2.1 ± 1.18 *N. variatella* per trap and the water baited filter paper control catching no *N. variatella* males (Fig. 7).

N. variatella in strip sprayed units. In strip sprayed units, we found no significant differences ($F = 0.47$, $df = 5$, $P = 0.7966$) in trap catch of *N. variatella* males along the transect. However, traps in strip sprayed units caught a mean of 13.60 ± 1.25 *N. variatella* per trap per wk, significantly fewer moths ($F = 14.00$, $df = 1$, $P = 0.0003$) than the mean of 48.86 ± 7.28 *N. variatella* per trap per wk caught in adjacent untreated units. We found a significant difference ($F = 6.03$, $df = 5$, $P = < 0.0001$) in *N. variatella* trap catch over time in strip sprayed units (Fig. 8). *Nemapogon variatella* trap catch steadily increased for three wks following a pheromone application (7 July 2006), but after the next pheromone application (30 July 2006) halfway through the fourth wk trap capture was suppressed to a rate equivalent to the first wk (Fig. 8).

Discussion

***P. robiniae* Spatial Distribution.** Our trap density study showed a “bowl” type distribution of trap capture of *P. robiniae* males in a 16 ha block with 40 traps positioned at one trap per 0.4 ha (Fig. 1). This bowl shaped distribution pattern suggest one of two

things: 1) either male *P. robiniae* are following a pheromone plume from inside the block until they reach clean air outside the block and then turn around and find the nearest pheromone source (Kennedy and Marsh, 1974), which would be an outside (border) trap; or 2) male *P. robiniae* from outside of the 16 ha block are being attracted to the pheromone and are being caught in the outside traps. Wedding et al. (1995) found that the outside traps caught more *Neodiprion sertifer* (Geoffroy) (Hymenoptera: Diprionidae) (European pine sawfly) males than interior traps in grids of 6x6 traps baited with female sex pheromone. Mark and recapture studies in the same grid experiment showed that the majority of *N. sertifer* males originated from outside of the grid (Wedding et al. 1995). We do not know whether *P. robiniae* males caught in our outside traps are being attracted from outside the area or emigrating toward clean air from interior locations where they then turn around to find the nearest pheromone source. However with the large *P. robiniae* population in 2003, it was likely that both scenarios were occurring at the same time. Either scenario could be considered camouflage of the females' natural pheromone plume or competition between calling females and multiple point sources of synthetic pheromone (Carde and Minks 1995) and would produce the results we recorded.

Anecdotally, on farms where the pheromone control strategy targeted specific units and complete saturation of *P. robiniae* pheromone was not implemented, we found more damage occurred on the edge of these treated units. More damaged apples occurring on the outside trees than the interior trees has also been found with mating disruption of codling moths in orchard systems too (Brunner et al. 2002). The bowl shaped distribution of male *P. robiniae* in our trap density study could explain why we find more damage on the edges of these pheromone treated units. By only applying

pheromone to single units, the male population is increased on the borders of these units because of one or both of the scenarios presented above, increasing the chance of successful mating of females in these border areas, thus damage occurs on edge trees. Pearson (1995) reported a higher infestation rate of squash vine borer (*Melittia cucurbitae* (Harris)) (Lepidoptera: Sesiidae) in squash fields treated with female sex pheromone. The squash fields are rotated yearly to prevent a build up *M. cucurbitae* populations, so females need to immigrate to find suitable oviposition sites (Pearson 1995). Pearson (1995) also states it is not known whether females *M. cucurbitae* mate then immigrate or immigrate then mate in squash fields. If *M. cucurbitae* females immigrate to squash fields and then call for males, a pheromone treatment on these small plots (76 m²) with the female sex pheromone could attract a greater number of males to that area, increasing the likelihood of mating. Pearson (1995) did not discuss this option, which we think is a possibility for the patterns we have seen in *P. robiniae* populations (Fig. 1).

Pheromone Drift. *Adjacent Block.* On Potlatch's South farm we were able to look at the relationship between pheromone treated units and how pheromone drift affected trap catch on neighboring untreated units. We saw a significant difference in trap catch between untreated units that had no contact with treated units compared to untreated units that were in contact with treated units. Although there were no significant differences between the numbers of treated units bordering an untreated unit (1, 2, or 3) there was decreasing trap capture of moths with increasing contact with treated units. The differences we recorded in trap catch from wk to wk could be attributed to the phenology of *P. robiniae* and not to the number of bordering units treated with

pheromone because we found no wk by treatment interactions. The lack of wk by treatment interaction effects suggests that pheromone drift affects differing population sizes proportionately.

Downwind Traps. Our results show that traps in untreated units directly downwind of pheromone treated units caught significantly fewer male *P. robiniae* than traps positioned directly upwind of pheromone treated units. These data also suggest that pheromone drift from a treated unit directly upwind affects trap capture of male *P. robiniae* more reliably than randomly positioned bordering units that might be upwind or perpendicular to treated units. In a similar study, there were no differences in trap capture of *N. sertifer* found in relation to wind direction, however increases in wind velocity caused a higher proportion of *N. sertifer* males to be caught in upwind traps, suggesting more consistent pheromone plumes with higher velocity winds (Wedding et al. 1995). Here too, males within treated units cannot find pheromone baited traps, their continued upwind flight may take them to clean air, where they can locate and be captured in traps located upwind of the treated units.

Farms isolated from pheromone. On the North farm trap capture seemed to be significantly affected by each pheromone application made on the South farm. The North farm, although untreated, differed from the also untreated Wallula farm in two aspects: 1) The North farm was closer to pheromone treatments than Wallula, <5.6 km compared to >7.4 km respectively, and 2) The North farm is situated downwind from pheromone treated units while the Wallula farm is positioned upwind. The Wallula farm was >56 km downwind from any pheromone application. A combination of position in respect to

pheromone treated units and distance from pheromone treated units influenced how pheromone drift affected untreated units in these two farms.

Bergh et al. (2004) reported inconsistencies with commercially available pheromone lures for *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae). When a specific *S. scitula* pheromone was formulated (Zhang et al. 2005), it was found to be extremely attractive, catching up to 28 times more males than traps baited with virgin *S. scitula* females, and more attractive than any combination of the components reported by Bergh et al. (2004). The sex pheromone for *P. robiniae* was synthesized by Cowles et al. (1996), so having a non-species-specific pheromone has not been a problem. Since 2002, we have been observing large numbers of *P. robiniae* male moths caught in pheromone baited traps (>108,000 *P. robiniae* in an 18 wk season) (Brown et al. 2006).

The attractiveness of *P. robiniae* males to the female sex pheromone, and the results reported above, have lead to the implementation of strip spraying on several farms (Kittelson et al. Unpublished). Strip spraying involves applying pheromone to one third of a unit and relying on residual pheromone and pheromone drift to cover the remaining two thirds of the unit. If pheromone drift can adequately protect the remaining two thirds of a unit, this will provide economic relief to poplar growers by reducing the amount of pheromone used and reducing the cost of aerial applied pheromone applications by two thirds. Our strip spraying regime consists of 30 m wide treated strips followed by two 30 m (60 m) untreated strips with another 30 m treated strip following sequentially. Sharov et al. (2002) reported trap catch of male gypsy moth (*Lymantria dispar* (L.)) (Lepidoptera: Lymantriidae) was affectively reduced and consequently fewer mated females were found from 150 m to 250 m outside of pheromone treated areas. The

report of reduced mating success outside of treated areas has only been validated in areas of low population density, similar tests in highly populated areas have not shown any reduction in mating success of female *L. dispar* (Sharov et al 2002). Tcheslavskaia et al. (2005) reported significantly fewer male *L. dispar* caught within their strip sprayed units than outside of the strip sprayed units. Similar to our method, Tcheslavskaia et al. (2005) used 30 m strips, however in between treated strips there was 90 m of untreated area. Tcheslavskaia et al. (2005) also reported reduced mating success of females within a strip sprayed area, regardless of location (in the 30 m treated strips or the 90 m untreated strips). In our strip sprayed units the furthest untreated area is only 30 m from the nearest treated strip. Strip spraying should only be implemented where there has been a history of low populations of *P. robiniae*. We have not been able to report any reduced mating success of females in these strip sprayed units, however, we have recorded continued reductions in *P. robiniae* damage in newly planted units within close proximity of strip sprayed units, which suggests a reduction of emigrating mated female *P. robiniae*.

***Nemapogon variatella*.** The fact that all of the *N. variatella* specimens were male and that the specimens were caught flying around a trap baited with *P. robiniae* sex pheromone suggest that these two different species of moths have female sex pheromones with similar components. There is no sex pheromone listed for *N. variatella* in the literature; however, Szöcs, et al. (1989) lists Z, Z- 3, 13-octadecadienyl alcohol (a 20% component of *P. robiniae* sex pheromone) as the pheromone for *Nemapogon personella* (Pierce & Metcalfe), which Gustafsson (2005) lists as a synonym of *N. variatella*.

Heinrich et al. (2000) lists various combinations of E, Z and Z, Z- 3, 13-octadecadienyl acetates and alcohols as pheromones for seven species within the *Nemapogon* genus.

There is little information on *N. variatella* in the literature, and none on the biology of the moth. Anonymous (2003) lists fungi and stored vegetable products as the host of *N. variatella*. The understory of hybrid poplar trees provide adequate habitat for fungi and *N. variatella* are found throughout the farm, and our data suggest that *N. variatella* have a similar flight period to *P. robiniae* (Fig. 6). Thus, with *N. variatella* being cosmopolitan on the farms and having relatively the same flight period as *P. robiniae*; increases in trap capture of *N. variatella* may indicate pheromone concentration may be inadequate to control *P. robiniae* in the absence of *P. robiniae* males to monitor.

With aerial applications of the flowable *P. robiniae* pheromone only being effective for four wks, and the implementation of strip spraying where certain areas are left untreated for a period of time; *N. variatella* could provide information useful for monitoring the pheromone's presence and potential effectiveness to disrupt *P. robiniae* mating. We propose that by following trap capture of male *N. variatella*, the relative flowable pheromone concentration could be monitored in the absence of *P. robiniae* males. To our knowledge, this would be the first use of a non-pest species to monitor the sex pheromone of a pest species. In other words, *N. variatella* is a "canary" species for the presence or absence of *P. robiniae* sex pheromone.

Effects of Pheromone on N. variatella. The results from our *P. robiniae* monitoring traps have shown that *N. variatella* are highly affected by the pheromone treatments for *P. robiniae* control. In units that were completely saturated with pheromone we caught no (0) *N. variatella*, whereas the monitoring traps in untreated

units had large populations of *N. variatella* in all the traps, some exceeding 1,500 *N. variatella*/trap/wk. However, after three years of using a pheromone-based strategy to control populations of *P. robiniae*, we still captured *N. variatella* in untreated units as well as strip sprayed units. Thus we have no evidence that *N. variatella* populations have been affected by extensive use of *P. robiniae* pheromone.

Varying Concentrations of Paranthrene robiniae pheromone. The commercial septum's release rate of *P. robiniae* pheromone probably exceeded the release rate of the microcapsules in the flowable formulation, and therefore caught more *N. variatella* males than the 1 mg of flowable pheromone loaded on to filter paper. The fact that the commercially bought septum caught more moths suggests that it should be effective at monitoring *N. variatella* even when flowable pheromone concentrations are high in treated areas. Our results from this study also demonstrated that trap capture of *N. variatella* was inversely proportional to the concentration of *P. robiniae* flowable formulation of pheromone on filter paper. If this method can be applied in pheromone treated units, i.e., if trap capture of *N. variatella* males increased as ambient flowable pheromone levels decreased over time, pheromone efficacy can be followed in the absence of *P. robiniae*. Thus in addition to the use of "super" lures (10 mg) baited traps to monitor the *P. robiniae* population, pest managers should be attentive to the number of *N. variatella* male moths being capture as well.

N. variatella in strip sprayed units. We found no difference in trap catch of *N. variatella* in the transect of traps within strip sprayed units in relation to trap placement. Similar to our results, Tcheslavskaia et al. (2005) reported significantly greater number of *L. dispar* moths captured outside of the strip sprayed unit than within. They also found

that significantly fewer male *L. dispar* were caught within the 30 m treated strips than within the 90 m untreated strips. We found no significant differences in trap capture of *N. variatella* males within our 30 m sprayed strips, when compared to traps located within our 60m untreated strips. Our results suggest that residuals from the previous month's pheromone application and drift from treated strips into untreated strips provides adequate pheromone coverage to shutdown trap catch of both *P. robiniae* and *N. variatella* throughout these units. We did find that trap capture of *N. variatella* in strip sprayed units varied significantly over time (Fig. 8). Our results show that *N. variatella* trap capture increased in the period preceeding pheromone applications and significantly decreased directly after the next pheromone application (Fig. 8). Trap capture in the control units was significantly higher than in strip sprayed units; however, over time capture rate in control units mirrored those of the strip sprayed units. Control units were interspersed with treated units and some were downwind of strip sprayed units; so *N. variatella* capture in these units may have been affected similarly to the drift affect shown in Fig. 2 for *P. robiniae*. These results suggest that with consistent monitoring of *N. variatella* in *P. robiniae* pheromone baited traps, pheromone levels can be monitored in the absence of *P. robiniae* males.

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Figures:

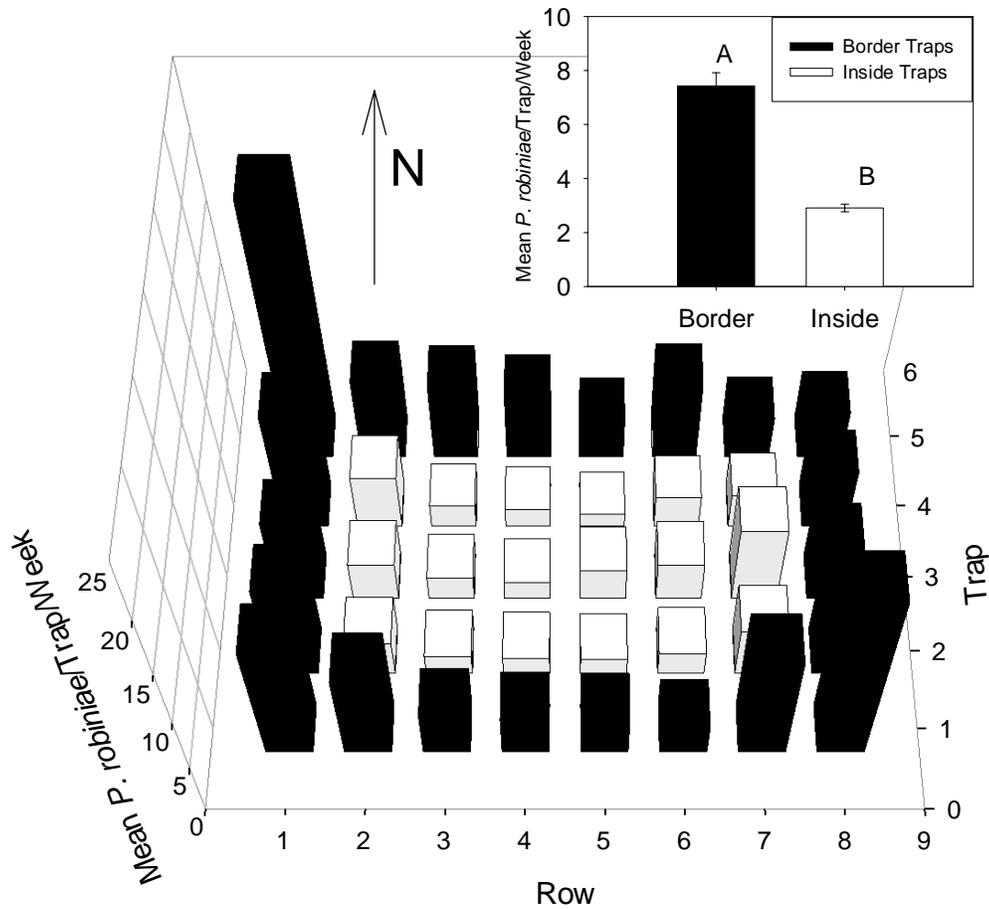


Figure 1: Capture from 16 ha blocks with traps spaced at one trap per 0.4 ha. This pattern was replicated in three independent units and trap capture was monitored for 12 wks. We found significant ($F = 2.65$, $df = 28$, $P < 0.0001$) row by trap interaction with border traps capturing significantly more moths than interior traps. Means with the same letters are not significantly different at $P < 0.05$.

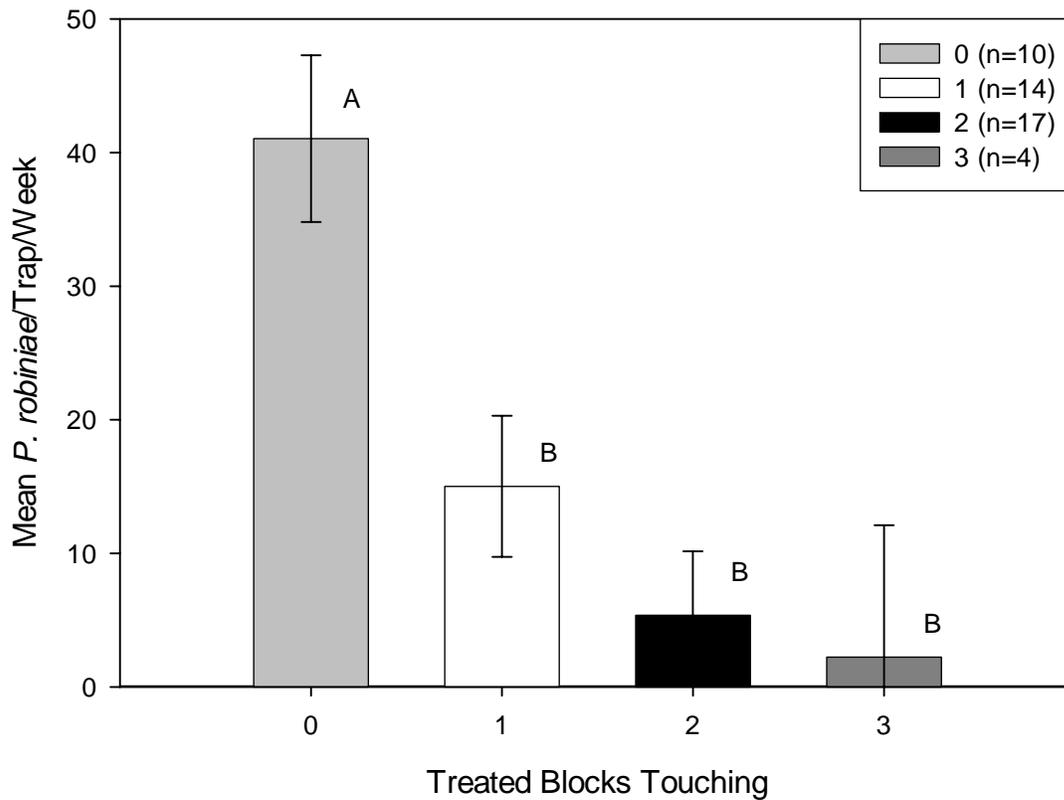


Figure 2: Trap capture of *P. robiniae* male moths at Potlatch in untreated units in respect to the treatment or non-treatment of neighboring units with *P. robiniae* sex pheromone from 1 June 2003 through the end of the season. Means with the same letters are not significantly different at $P < 0.05$, n = Number of units (65 ha each) monitored.

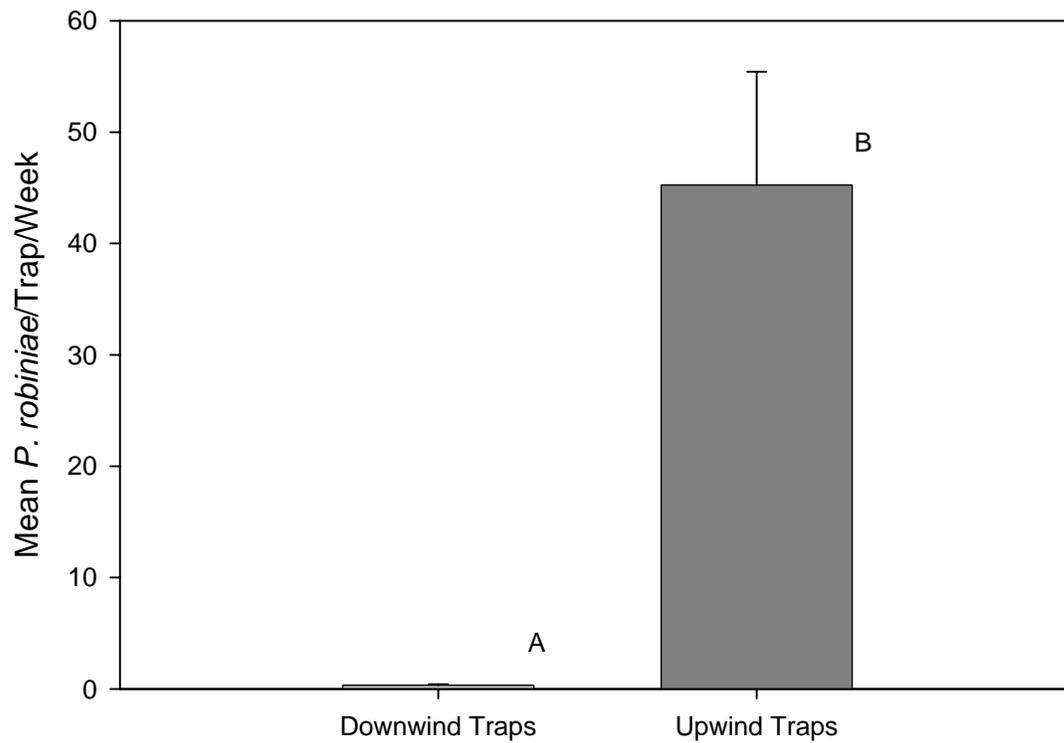


Figure 3: Trap capture of male *P. robiniae* at Boise in 2003 in traps positioned either directly upwind (210° to 240°)(n = 5) or directly downwind (30° to 60°) (n = 5) of pheromone treated units from 1 June 2003 through the end of the season. Means with the same letters are not significantly different at $P < 0.05$.

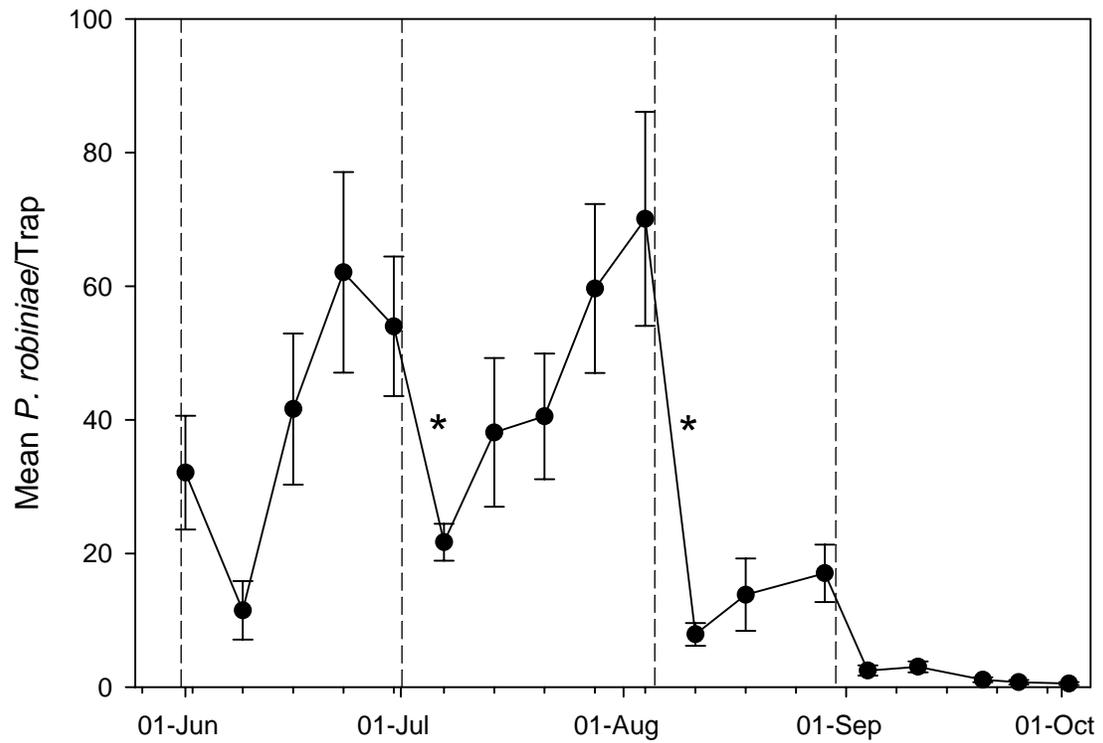


Figure 4: Trap capture (n = 29) of male *P. robiniae* on Potlatch's North farm in 2003. No pheromone applications were made on this farm in 2003. Dotted line represents the date at which pheromone applications were made on the South farm. The asterisks (*) represent a significant ($P < 0.05$) reduction in mean trap capture compared to the previous week.

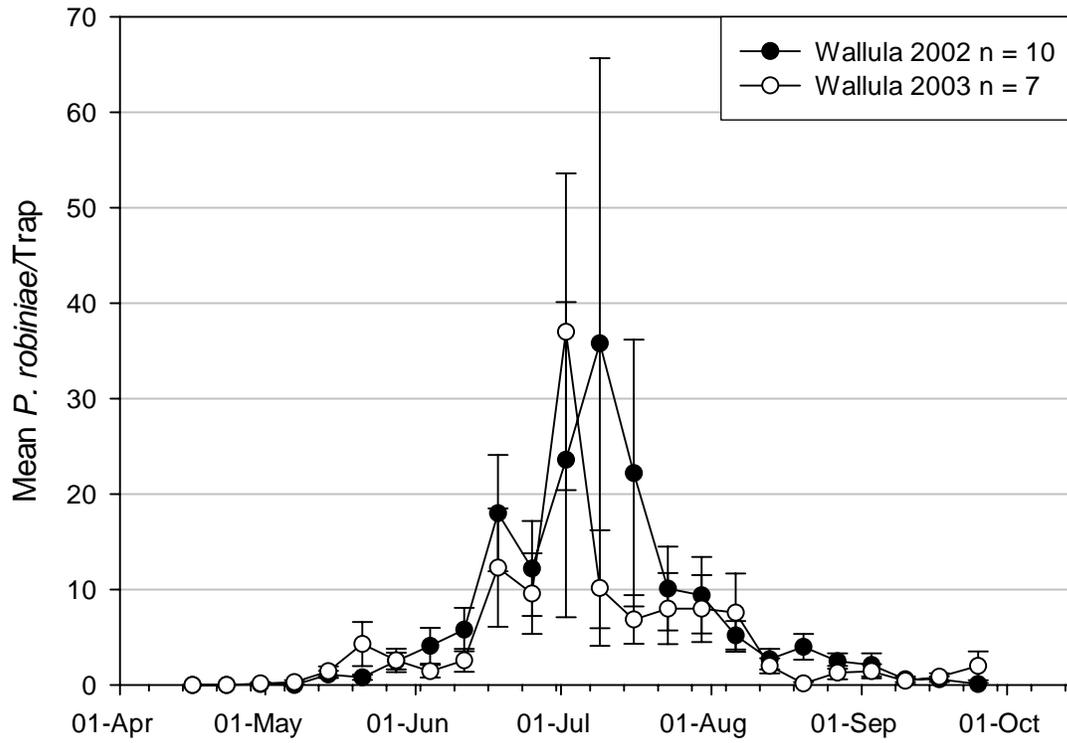


Figure 5: *P. robiniae* flight period with a single peak in the untreated Wallula farm (466.48 ha). n = Number of monitoring traps.

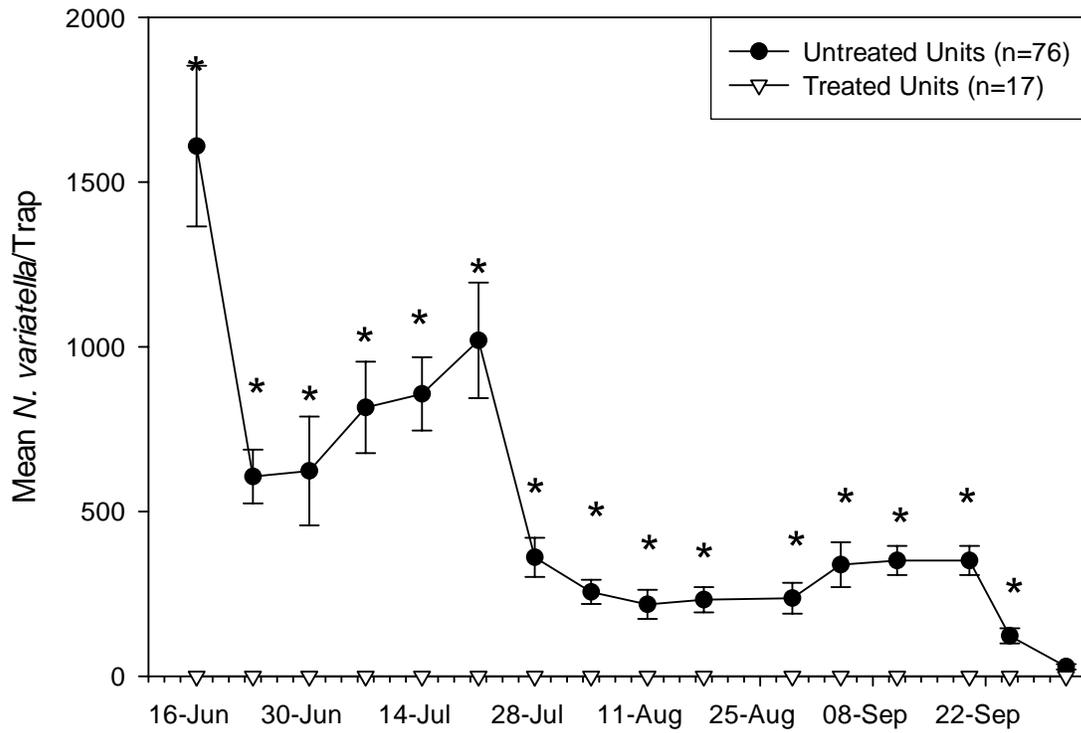


Figure 6: Trap capture of male *N. variatella* from Potlatch plantation in 2003. The asterisks (*) represent a significant ($P < 0.05$) difference in mean trap capture for that wk between treated and untreated units, each unit (n) represents either a 65 or 110 ha unit.

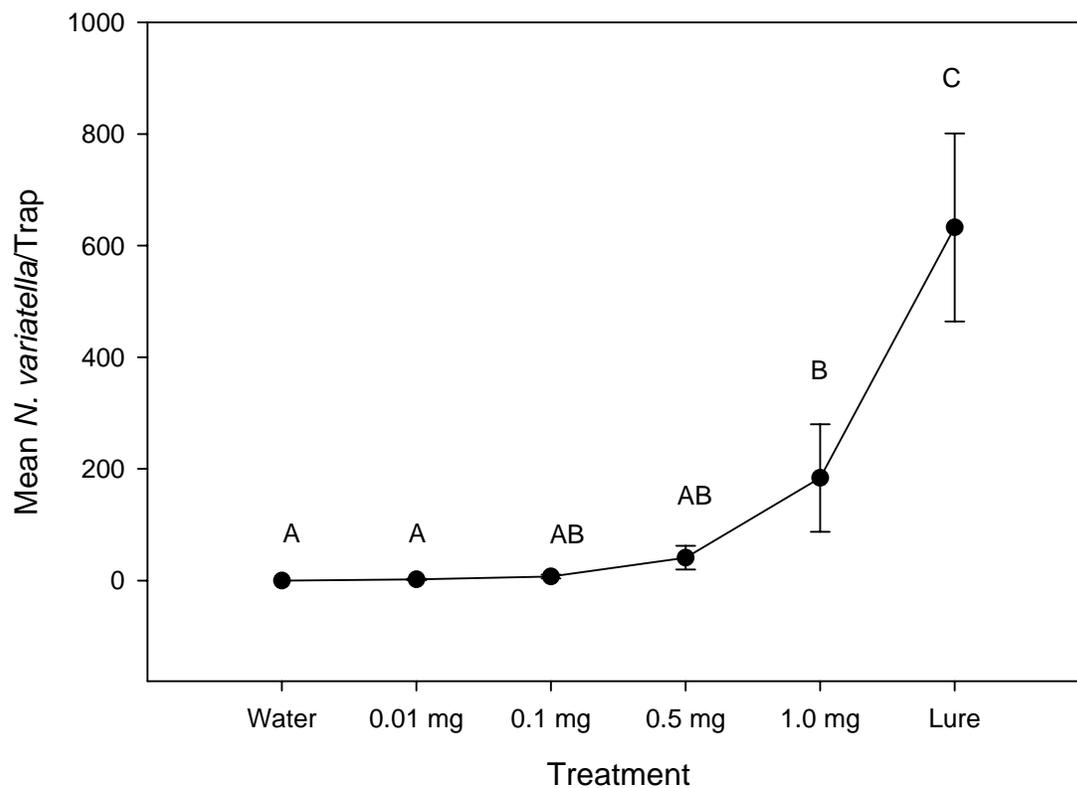


Figure 7: Trap capture of male *N. variatella* in bucket traps baited with various concentration (mg of active ingredient) of *P. robiniae* flowable pheromone on filter paper. Each replication (n= 11) was positioned in an independent unit. A commercial septum loaded with 1 mg of *P. robiniae* sex pheromone and filter paper with 1 ml of water served as upper and lower limit controls, respectively. Means with the same letters are not significantly different at $P < 0.05$.

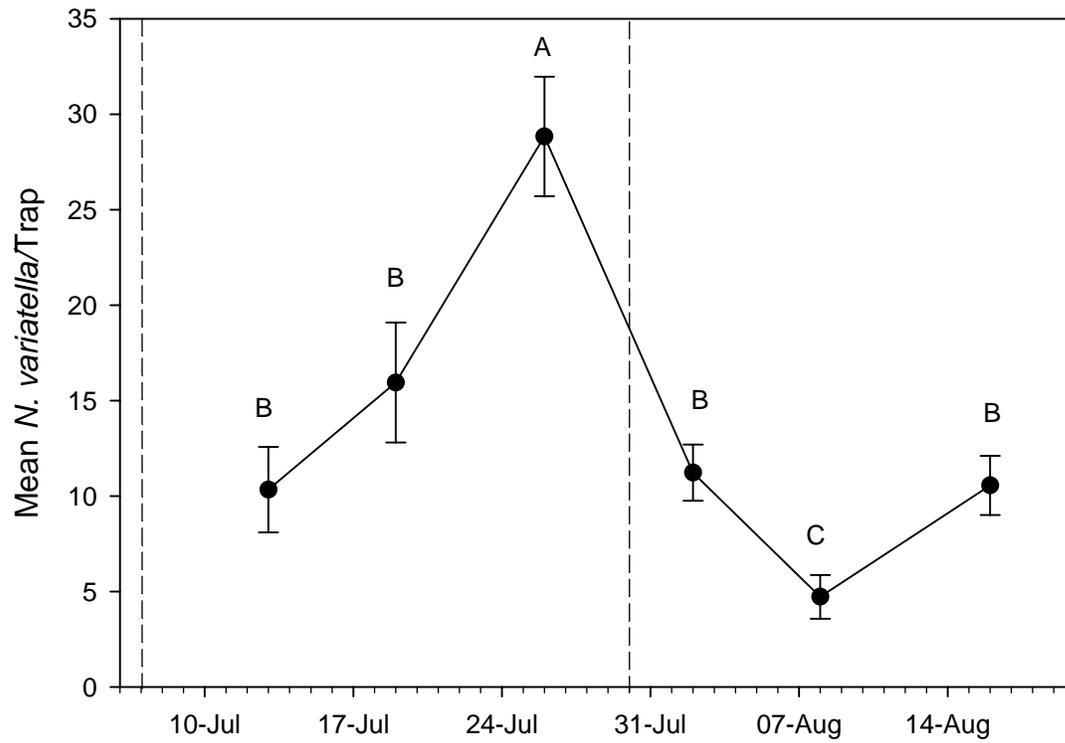


Figure 8: Trap capture (n = 18) of male *N. variatella* in units strip sprayed with *P. robiniae* sex pheromone. Dotted line represents a pheromone application. Means with the same letters are not significantly different at $P < 0.05$.

Chapter 6: Other clearwing moth species.

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ABSTRACT: Eleven species of clearwing moths attack trees in the Salicaceae family in North America. Only five of these species occur in the northwest and only three attack *Populus spp.* The only clearwing moths that we have captured in our monitoring traps in poplar plantations are *Paranthrene robiniae* (Hy. Edwards), for which we have a specific sex pheromone. Several different pheromone lures were purchased from Suterra LLC., and traps baited with these lures were monitored at several locations. We caught a total of three different species of clearwing moths in these traps, including *P. robiniae*. The other two were *Synanthedon sequoiae* (Hy. Edwards) (sequoia pitch moth), which does not attack poplar, and *Sesia tibialis* (Harris) (cottonwood crown borer).

Introduction

The sex pheromone for the western poplar clearwing moth is very specific to the species (Cowles et al. 1996). Lures for other species of clearwing moths are not so specific and some attract a variety of other clearwing moths besides the species being monitored. Neilsen et al. (1979) and Rogers and Grant (1991) have reported numerous species of sesiid caught with a 99% pure ZZ 3,13 octadecadien -1-ol acetate (ODDA) lure. With our lure being specific to *P. robiniae*, we were uncertain if there are any other clearwing moths in the hybrid poplar farms. According to Eichlin and Duckworth (1988), there are 11 species of clearwing moths that exploit trees in the Salicaceae family in North America. Three species in the *Paranthrene* genus: *P. dollii* (Neumoegen) (Doll's clearwing moth), *P. tabaniformis* (Rottemburg) (the dusky clearwing), and *P. robiniae* (western poplar clearwing moth) (Fig. 1). There are two species in the *Sesia* genus that attack trees in the Salicaceae family: *S. apiformis* (Clerck) (the hornet moth) and *S. tibialis* (Harris) (the cottonwood crown borer) (Fig. 2). The *Synanthedon* genus has five species that attack the Salicaceae family: *S. albicornis* (Hy. Edwards) (the willow stemborer) (Fig. 3), *S. bolteri* (Hy. Edwards) (no common name) (Fig. 4), *S. proxima* (Hy. Edwards) (the willow borer), *S. scitula* (Harris) (the dogwood borer), and *S. sigmoidea* (Beutenmüller) (no common name). Out of these 11 species only five occur in the northwestern USA: *P. tabaniformis*, *P. robiniae*, *Sesia tibialis*, *Synanthedon albicornis* and *S. bolteri*. *Synanthedon albicornis*, and *S. bolteri* have only been recorded on *Salix* species and are not known to attack *Populus* species. This leaves only three possible clearwings that attack poplar trees in our region, one of which we are already familiar with, *P. robiniae*; the other two being *S. tibialis* and *P. tabaniformis*.

Paranthrene dollii, *P. tabaniformis* and *P. robiniae* are considered sister species and their ranges basically divide North America in three sections. *Paranthrene dollii*'s range is in the eastern side of the North America from Texas in the south to Wisconsin in the north; *P. tabaniformis*' range covers the eastern and middle North America from the east coast to the Rocky Mountains, north into Alaska and south into Mississippi and Georgia (Eichlin and Duckworth 1988). *Paranthrene robiniae*'s range covers the western side of North America from the west coast to the Rocky Mountains, north into Alaska and to the south throughout California (Eichlin and Duckworth 1988). According to Eichlin and Duckworth (1988) the range of *P. robiniae* and *P. tabaniformis* range overlaps around the Rocky Mountains. The pheromone listed for *P. tabaniformis* is a 9:1 blend of EZ to ZZ -3,13-octadecadienyl straight-chain 18 carbon alcohols (ODDOH) (Karalius et al., 2001), while the pheromone for *P. robiniae* is a 4:1 ratio of EZ to ZZ-3,13-ODDOH (Cowles et al. 1996). With the main component for both moths being the same, it is likely *P. tabaniformis* would be attracted to a *P. robiniae* pheromone baited trap.

Given the fact that there are other clearwing moths that attack *Populus spp.* and the pheromone we are using for the western poplar clearwing moth is specific *P. robiniae*, we were curious to find out if there are other clearwing moths that we could trap and if any of these had the possibility of attacking hybrid poplars.

Materials and Methods

We purchased several different lures that were known to trap a variety of species of clearwing moths in other areas. Peachtree borer (*Synanthedon exitiosa*) pheromone (1:24 E,Z to Z,Z 3,13- ODDA), dogwood borer (*S. scitula*) pheromone (100 E,Z 2,13-

ODDA to 1 Z,Z -3, 13 -ODDA), and sequoia pitch moth (*S. sequoiae*) pheromone (100% Z,Z 3,13-ODDOH) lures were purchased from Suterra LLC (213 SW Columbia, Bend, OR), (Heinrich et al. 2000). We placed a set of three traps, each with a different lure at four locations: one set was placed inside Potlatch's North farm (45° 53' 36.74"N, 119° 34' 42.91"W), one set inside Greenwood Resources' Sand Lake farm (45° 42' 29.52"N, 119° 31' 36.76"W), a set inside a riparian area in the Umatilla National Wildlife Refuge (45° 53' 36.74"N, 119° 34' 42.91"W), and another at the Steffin's Center (Forestry Center, Washington State University Pullman Campus) (46° 44' 02.85"N, 117° 07' 42.44"W). These traps were checked every two wks from 15 June to 26 September 2005. Along with the described set of traps, six *P. robiniae* pheromone baited traps were also placed at the Steffin's Center.

Results

There were no (0) clearwing moths of any species other than *P. robiniae* caught in any of the traps baited with sex pheromones placed at Potlatch, Sand Lake or at the Umatilla National Wildlife Refuge. At the Steffin's Center we caught three different species of clearwing. We caught 16 *P. robiniae* (Fig. 1a), five cottonwood crown borers (*Sesia tibialis*) and 50 sequoia pitch moths (*Synanthedon sequoiae* (Fig. 5 and 6).

Discussion

The main pheromone component for all *Paranthrene* species that attack poplar is EZ 3,13 -octadecadienyl straight-chain 18 carbon alcohol (ODDOH). *Paranthrene dollii*'s pheromone is a 3:1 EZ to ZZ 3,13 ODDOH (Nielsen et al. 1979); for *P. robiniae*, the pheromone consists of 4:1 EZ to ZZ 3,13 ODDOH (Cowles et al. 1996); and *P. tabaniformis* has been trapped with 9:1 EZ to ZZ 3, 13-ODDOH (Karalius et al. 2001). It

is likely that with the similarity of their sex pheromones, if other clearwing moths from the *Paranthrene* genus were present in the hybrid poplar plantation, we would have found them in our traps baited with the 4:1 E,Z: Z,Z ODDOH blend. Pheromone blends escape the rubber septa at different rates, a lure loaded with a 4:1 ratio of EZ:ZZ ODDOH could be emitting a 3:1 ratio after several wks, thereby possibly attracting *P. dollii*. The scientific data on evaporation rate of the isomers is unknown at this time. *Paranthrene dollii* and *P. tabaniformis* have different visual characteristics (Fig. 1b, c) that would allow us to recognize them from *P. robiniae* with little effort. It is highly unlikely that *P. dollii* would be found in the northwest since its range is in the east, but finding *P. tabaniformis* in the northwest would not be surprising especially on the east side of Oregon or Washington where *P. tabaniformis* and *P. robiniae*'s range may overlap.

Sesia tibialis, the cottonwood crown borer, is the other clearwing moth we caught that does attack poplar trees. In total we only captured five *S. tibialis* at the Pullman, WA location; this may be due to sub-optimal lures, time of the year, or we were trapping in a small population. No *S. tibialis* were found on any of the hybrid poplar farms or at the Umatilla National Wildlife Refuge. The known pheromone components of *S. tibialis* are 1 ZZ 3, 13 ODDOH to 4 ZZ 3, 13 ODDA (Underhill et al. 1978). We captured *S. tibialis* in traps baited with *Synanthedon exitosa* (peachtree borer) pheromone (various ratios of EZ to ZZ 3,13 ODDA)(Bergh et al. 2004) and *Synanthedon scitula* (dogwood borer) pheromone (100 EZ 2,13 ODDA to 1 ZZ 3, 13 ODDA) (Snow et al. 1985). Recently the pheromone for *S. scitula* has been refined, prior to Zhang et al. (2005) and Leskey et al. (2006), the commercial lure for *S. scitula* was 100 EZ 2, 13 ODDA to 1 ZZ 3,13 ODDA (Snow et al. 1985, Zhang et al. 2005). Although *S. tibialis* has one pheromone component

(E,Z 3, 13 ODDOH) similar to *P. robiniae*, we have never caught any cottonwood crown borers in *P. robiniae* monitoring traps, which suggests the ODDA main component is more important for *S. tibialis*, or there are no crown borers in our research area. We plan to bait some traps using the ODDA formulation in 2007. *Synanthedon exitosa* and *S. scitula* pheromones probably are not the best for monitoring *Sesia tibialis*. We recommend monitoring for *S. tibialis* with a more specific pheromone blend (Underhill 1978) that contains 20% alcohol (ZZ 3, 13 ODDOH) to 80% acetate (ZZ 3, 13 ODDA). At this time however we do not think *S. tibialis* is a problem, since none were caught on the poplar farms or the wildlife refuge. Secondly, if *S. tibialis* were present on the farms in large enough populations to be of concern, the damage would be evident in our damage surveys, because damage from *S. tibialis* has been described as similar to that of *P. robiniae* (Dreistadt and Perry 2004). If *S. tibialis* were unaffected by our pheromone control strategy, because of the pheromone differences, we would mistake *S. tibialis* damage for *P. robiniae* damage, thus making the pheromone control strategy seem less successful. However, considering the continued reduction in our damage surveys, and the lack of *S. tibialis* from trapping, we do not believe they are a problem at this time.

Synanthedon sequoia (sequoia pitch moth) was the other species we caught at the Steffin's Center. The sequoia pitch moth does not attack *Populus* spp; its hosts are primarily pine species. We knew from previous trapping and the historical record in the M. James Insect Collection at WSU Pullman, that this species is present in our area. The sequoia pitch moth also has a similar pheromone (Z,Z 3, 13 – ODDOH) to the western poplar clearwing moth (Nielsen and Purrington 1978a, b). Figure 6 shows that four sequoia pitch moths were caught in western poplar clearwing moth pheromone baited

traps, however there were no western poplar clearwing moths caught in sequoia pitch moth pheromone baited traps. The fact that no *P. robiniae* were caught in sequoia pitch moth traps shows how selective or sensitive *P. robiniae* males are to their own pheromone, considering the close proximity of trap locations and the similar pheromone blends of the two moths. The pheromone for the western poplar clearwing moth is a 4:1 ratio of EZ to ZZ 3,13- ODDOH (Crowles et al., 1996), where as the sequoia pitch moth has been captured with ZZ 3,13 ODDOH (Nielsen and Purrington, 1978a, b).

The purpose of this chapter is to draw attention to the possibility of other clearwing moth species that may be found in the hybrid poplar farms or caught in monitoring traps baited for *P. robiniae*. We have never caught any other clearwing moths in monitoring traps baited with the sex pheromone of the western poplar clearwing moth, but there is a possibility other moths are there. We feel that none of these other species pose a threat at this time. Monitoring for *S. tibialis* periodically should be continued. *Paranthrene tabaniformis* would probably show up in traps baited with *P. robiniae*'s sex pheromone, considering its pheromone (9:1 EZ to ZZ 3, 13-ODDOH) similarities to *P. robiniae* pheromone (4:1 EZ to ZZ 3, 13-ODDOH). Also because of pheromone similarities, if *P. tabaniformis* is susceptible to *P. robiniae*'s pheromone, it may also be susceptible to our pheromone control strategy.

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Figures:



Figure 1: A: *Paranthrene robiniae*, B: *Paranthrene tabaniformis*, C: *Paranthrene dollii*. All three are known to attack Salicaceae species. Photo Credits (A: James Solomon, USDA Forest Service B: Gyorgy Csoka, Hungary Forest Research Institute C: James Solomon, USDA Forest Service).



Figure 2: *Sesia tibialis*, the cotton wood crown borer, is known to attack poplars (Bruce Neill, Agriculture and Agri-Food Canada).



Figure 3: *Synanthedon albicornis*. (the willow stem borer). (C. Henne, LACM). *S. albicornis* is known only to attack *Salix spp.* within the Salicaceae family.

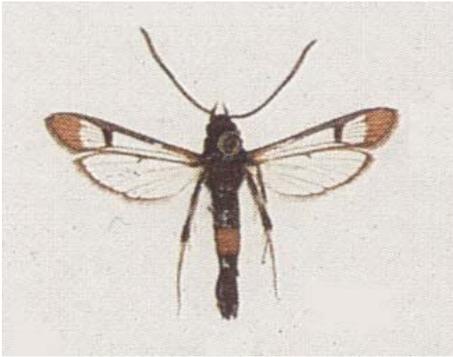


Figure 4: *Synanthedon bolteri* (J.D. Ritchie, USNM). *S. bolteri* is known only to attack *Salix spp.* within the Salicaceae family.



Figure 5: *Synanthedon sequoiae*, the sequoia pitch moth, does not attack poplars (Jerald E. Dewey, USDA Forest Service).

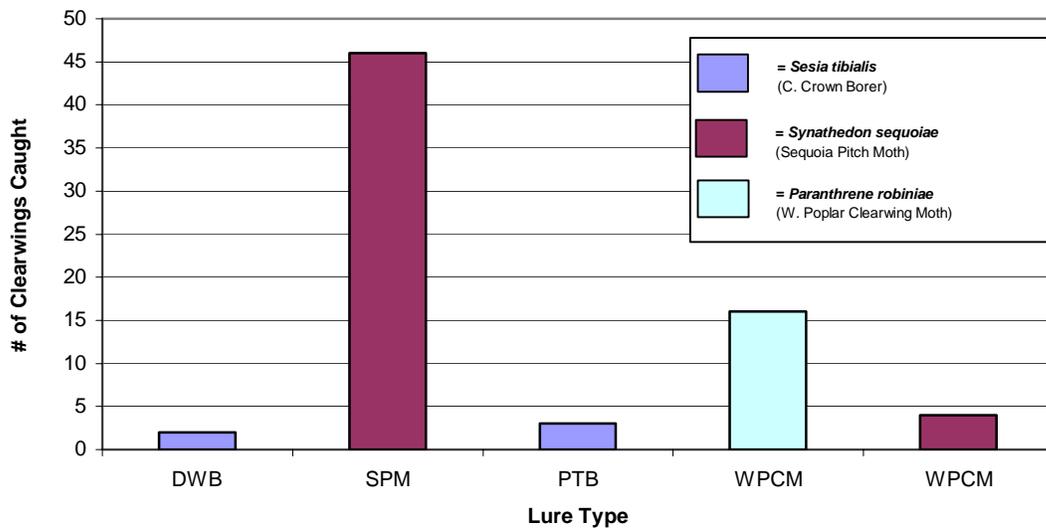


Figure 6: Results from pheromone baited trap catch at the Steffin's Center, showing which lure type caught which moth and how many of each species of clearwing were captured. Lure types: DWB = dogwood borer, SPM = sequoia pitch moth, PTB = peachtree borer, and WPCM = western poplar clearwing moth).

Chapter 7: *Prionoxystus robiniae* Cellulases

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ABSTRACT: Xylophagous insects seldom have cellulases capable of metabolizing cellulose; research has reported protozoans, yeasts, and fungi to be responsible for digesting the plant cellulose for most insect species inhabiting the heartwood of trees. We initiated a laboratory study to investigate if larvae of the carpenterworm moth (*Prionoxystus robiniae*, referred to as *Pr. robiniae*) have cellulases in their digestive system.

Our preliminary data do not support an endogenous source of cellulases in this Lepidoptera species. What would this mean if it were proven that *Pr. robiniae* larvae rely upon a microbes source for cellulases? Literature does not support transfer of symbiotic microbial through the parent to the progeny, therefore newly hatched *Pr. robiniae* larvae probably need to ingest fungal spores, or excavate and feed within borrows infested with an active fungal agent. If this were so, an application of a fungicide may disrupt the life cycle of these moths that are relying on microbial cellulases.

Introduction

In a tree there is an abundance of “non-living” tissue in the trunk (xylem) that seems relatively unused by phytophages compared to the rest of the tree (Taiz and Zeiger 2002). The xylem tissue is used less by insects because it is mostly made up of cellulose, which makes digestion of this material difficult (Buchner 1965, Henry 1967, Watanabe and Tokuda, 2001). Nonetheless there is a small guild of insects that are able to exploit this abundant material. Most of them are not able to exploit the xylem material alone though. Typically these so called xylophagous (feeding on xylem) insects need help from fungi or yeasts that produce cellulases, in order to digest the cellulose (Watanabe and Tokuda, 2001). Still other xylophagous insects such as termites and cockroaches can produce cellulases themselves (Watanabe and Tokuda, 2001). However, there is no information indicating that xylophagous Lepidoptera use cellulases from fungi or yeasts in the digestion of xylem material or that they can produce endogenous (self-produced) cellulases. The only mention of cellulases in lepidoteran digestion in the literature was reported by Teo et al. (1990), where they found only weak β -Glucosidase activity in the midgut. How xylophagous Lepidoptera are able to exploit the cellulose rich xylem tissue is unexplained.

Cellulose is a chain of glucose molecules joined together by β -1,4 linkages. To fully digest cellulose into usable glucose, a complex of three cellulases are needed. Cx-Cellulases (Endo- β -1,4-glucanase), randomly hydrolyzes within the cellulose chains. C₁-Cellulases (exo- β -1,4-cellobiohydrazase) hydrolyzes the non-reducing end of cellulose chains by cleaving off cellobiose units. Cellobiases (β -Glucosidase) hydrolyzes glucose

units from the non-reducing end of cellobiose or longer chain polymers (Kukor and Martin, 1986a, Watanabe and Tokuda, 2001).

Through a series of assays we planned to investigate, and hopefully determine whether *Prionoxystus robiniae* (Peck) moths produce their own cellulases or get them from an exogenous source. Kukor and Martin (1986a) found that *Monochamus marmorator* Kby, a cerambycid, acquired cellulases necessary for cellulose digestion from feeding on fungal infested wood. *Saperda calcarata* (Say), another cerambycid, when fed a cellulase enriched diet, was then able to digest cellulose (Kukor and Martin 1986b). If the cellulases in *Pr. robiniae* are shown to be produced exogenously, we then planned a follow up study with a feeding assay used by Kukor and Martin (1986b). The objective in the feeding assay would be to determine if the moths acquire the cellulases from a secondary source such as a xylophagous fungus that is developing on the tree.

The gut of *Pr. robiniae* was assayed as a whole, with and without gut content, to find what cellulases are present and where the cellulases are located. If cellulases were found in the gut tissue, the gut tract would then be separated into foregut, midgut and hindgut. These sections along with the gut contents and the salivary glands would be assayed separately in order to isolate where the enzymes are being produced. These tissues are the ones that produce the digestive enzymes in other xylophagous insects such as termites and cockroaches (Watanabe and Tokuda 2001). If enzymes showed up in the tissue extracts, that would indicate that the larva produces the enzymes endogenously. If the enzymes show up in the gut content and not the tissues, that would suggest that the enzymes are produced by something else, such as symbionts, or that they are acquired via ingestion, such as by eating fungus that contributes cellulases.

Materials and Methods

Collection of larvae: Larvae for digestive enzyme assays were actively feeding and collected from their natural habitat. Trees that were highly infested with *Pr. robiniae* were harvested and larvae were collected by destructive sampling. The larvae were then transferred to cold storage (-80°C) until assays were performed.

Dissection and Extraction: The larva's whole gut tract including contents was removed for the assays. The salivary glands were also removed for assays (Fig. 1). The gut was assayed as a whole, with and without gut content.

Extracts were prepared by dissecting the gut and/or the salivary glands from 10 larvae under cold deionized water (DI H₂O). Tissues and contents were stored in cold (ice bath) acetate buffer (0.2 M, pH 5.0) until dissections were complete. Combined tissues were homogenized by hand in either 2.5 ml (C_x and C₁ cellulases) or 5 ml (cellobiase) of cold acetate buffer (0.2 M, pH 5.0). The extract was then centrifuged for 20 min at 10,000x gravity (g) at 4°C, the supernatant was removed and saved at 4°C. The tissue pellet was re-homogenized by hand and suspended in 2.5 ml (C_x and C₁) or 5 ml (cellobiase) of cold acetate buffer (0.2 M, pH 5.0) and centrifuged again as above. The two supernatants were then combined. The extracts for the C_x and C₁ cellulase were brought up to 15 ml with cold acetate buffer (0.2M, pH 5.0) then filtered through an Amicon® filter (Amicon Corp, Danvers, MA) using nitrogen at 55 psi until 40 ml of cold acetate buffer (0.2M, pH 5.0) was pushed through at 10 ml increments. The extract was filtered through the Amicon® filter to remove glucose or equivalents from the extract prior to incubations so when assayed any color change present would be due to enzyme

activity and not residual glucose. Five (5) ml of the concentrate was used as the final extract. The filtrant was then tested for glucose equivalents. One ml of the filtrant was combined with 1 ml of 3,5-Dinitrosalicylic Acid (DNSA) reagent and boiled for 5 min. Absorbance was then read at 540 nm (Biomate 3 Spectrophotometer, ThermoSpectronic, Rochester, NY). Cold acetate buffer (0.2 M, pH 5.0) combined with 1 ml of DNSA reagent boiled for 5 min was used as the blank.

Enzyme Assay: *C_x-Cellulases (Endo- β -1, 4-glucanase)*. The C_x-activity (activity against carboxymethylcellulose) was determined by measuring the rate of liberation of reducing groups (maltose and equivalents) from the substrate (carboxymethylcellulose). An aliquot of extract in a total volume of 0.5 ml of acetate buffer (0.2 M, pH 5.0) was combined with 0.5 ml of 0.5% substrate solution and incubated at 37°C for 30 min. Adding 1 ml of DNSA reagent and boiling for 5 mins terminated the incubation. Then 1.5 ml of DI H₂O was added and the absorbance was measured at 540nm. Controls were run using boiled inactivated aliquots of the extract (Martin and Martin 1979).

C₁-Cellulases (exo- β -1, 4-cellobiohydrolase). The C₁-activity was measured using a modification of the above procedure. An equivalent of one gut tissue was held in 0.5 ml of cold acetate buffer (0.2 M, pH 5.0) used as an aliquot. This aliquot was combined with 50 mg of powdered cellulose and incubated at 37° C for 30 min. The mixture was then filtered through a sintered glass filter; the incubation tube was rinsed with 0.5 ml of DI H₂O and filtered also. The incubation was then terminated by adding 1 ml of DNSA reagent and boiled for 5 min. Then 1 ml of DI H₂O was added and absorbance was read at 540 nm. Controls were run using boiled inactivated aliquots of the extract (Martin and Martin 1979).

Cellobiases (β-Glucosidase). Cellobiase activity was determined by the release of p-nitrophenol from p-nitrophenyl- β-D- glucopyranoside. Assay mixtures consisted of an equivalent of one tissue in a volume of 1 ml cold acetate buffer (0.2 M, pH 5.0), and a 1 ml volume (3.32 mM) of the substrate solution in acetate buffer (0.2 M, pH 5.0). The mixture was incubated for 30 min at 37° C. The incubation was terminated by adding 2 ml of 1.0 M NH₄OH/NH₄Cl buffer (pH 10.0). Absorbance was then read at 460nm. Controls were run using boiled inactivated aliquots of the extract (Martin and Martin 1979).

Standard Curves. Standard curves to determine the units of cellulase activity of gut extracts were assayed each time an enzyme assay was run. *Trichoderma reesei* cellulase (CAS 8546) was used as the enzyme. Serial dilutions of 200, 150, 100, 50, 25, 10, 1 and 0 units/ml were made. One ml of cellulase was added to 1 ml substrate solution for each enzyme test. For Cx cellulases, 0.5 ml of 0.5% Carboxymethylcellulose was used as a substrate, for C1 cellulases, 50 mg of powdered cellulose in 0.5 ml of DIH₂O was used as the substrate and for Cellubiase, 1ml of P-nitrophenyl- β-D -glucopyranoside (3.32mM) in acetate buffer (0.2M, pH 5.0) was used for the substrate.

Reduced Carbohydrates in Poplar. We noticed a variation in poplar wood between where we extracted *Pr. robiniae* larvae and areas of the trunk where there were no larvae present. We cut out approximately 8 cm by 8 cm units of wood from the trunk of the tree where *Pr. robiniae* larvae had infested (infested) and from areas where no larvae were found (clean) with a Craftsman® (Sears, Hoffman Estates, IL) radial arm saw. Blocks were cut from three trees, seven units each of “infested” and “clean” were taken from tree one, three units of each were taken from both the second, and third tree.

Liquid was extracted from the wood units by pressing them to height of 1.5 cm in a Baldwin® 330,000psi press (Baldwin-Lima-Hamilton Corp, Philadelphia PA). Liquid was collected in a disposable aluminum pie tin and then transferred to a glass vial with a disposable transfer pipette. New pie tins and pipettes were used for each sample to avoid contamination. Liquid from both infested and clean wood was then assayed for reduced cellulose.

Assay. One ml of liquid (extract) from each block was centrifuged at 15,000 x g for 20 min. The extract was then combined with 1 ml DNSA reagent, and boiled for 5 min. After boiling, 2 ml of DI H₂O was added and absorbance was read at 540 nm. The blank for the colorimetric reading was 1 ml acetate buffer (0.2 M, pH 5.0) and 1 ml DNSA reagent. A standard curve was made using concentrations of glucose from 0.0001 g/ml to 0.001 g/ml in acetate buffer (0.2 M, pH 5.0) (1ml) and 1 ml DNSA reagent (Fig. 2).

Acquired Fungal Enzymes: Insects that do not produce cellulases endogenously and that do not have a symbiosis with a microbial xylophage are believed to acquire their cellulases by ingesting xylophagous fungi and utilizing their enzymes (Kukor and Martin 1986a). The following sections describe procedures for assessing larvae for the utilization of acquired fungal enzymes in their diet.

Diet: Larvae were fed one of two diets. Diet # 1 consisted of autoclaved clean poplar wood. Diet # 2 consisted of infested poplar. Diets consisted of 60g of either clean or infested poplar shavings, 500 ml of H₂O and 12.5 g of agar. Clean poplar shavings were autoclaved to ensure no active fungal enzymes were present in the diet. Infested shavings were not autoclaved. The 12.5 g of agar was added to the 500 ml of H₂O and

autoclaved. Clean shavings were added to the mixture, mixed, and allowed to set. The agar-water mixture was allowed to cool to <45°C before adding the infested shavings to avoid heat deactivation of any fungal enzymes present. Individual laboratory reared *Pr. robiniae* larvae were given approximately 2 g of the final agar-water-shaving mixture. Samples of each diet were assayed for the presence of all three cellulases.

Two groups of larvae were allowed to feed upon on each diet for a period of one week. After one week larvae from each group were assayed for cellulase enzyme activity. If larvae used acquired fungal enzymes to digest cellulose then the assay from the group fed diet # one should yield enzyme activity, while the assay from those fed diet # two should not.

pH of *Pr. robiniae* gut contents. Litmus paper tests suggested that the pH of *Pr. robiniae* gut contents were high. To test pH further, we used a pH indicating dye in the diet to determine the approximate pH of *Pr. robiniae* gut contents. Thymol blue (Thymolsulfonphthalein) dye was added to a general codling moth diet (Howell 1970) to make a 0.05% thymol blue diet. Seven larvae were allowed to feed on this diet for three wks. After the three wks larvae were dissected and guts were photographed to compare color to a standard curve. The standard curve was prepared using acetate buffer (0.2 M, pH 3.0) and NaOH buffer (0.5M pH 12.5). The two buffers were mixed to give us a gradient of pH's from 3 to 10, after which, thymol blue was added to make the solution 0.05%. Capillary tubes were filled with samples from each pH and photographed for comparison to guts.

Results

Enzyme Assay. We found activity from all three cellulases (C_x , C_1 , and cellulase) in the whole gut with contents (Table 1). When the gut tissues and gut contents were separated, only the gut content showed cellulase activity (Table 1), which suggests cellulases were acquired from an external source.

Reduced Carbohydrates in Poplar. We found a significant ($F = 12.89$, $df = 1$, $P = 0.0018$) difference in the amount of reduced carbohydrates in “infested” and “clean” poplar wood (Fig. 3). Samples taken from infested wood had a mean of $8.74 \times 10^{-4} \pm 1.99 \times 10^{-4}$ g carbohydrates/ml while the clean wood had only a mean of $2.94 \times 10^{-4} \pm 1.05 \times 10^{-5}$ g carbohydrates/ml. We also found a significant ($F = 3.91$, $df = 2$, $P = 0.0368$) difference in the amount of carbohydrates found in each tree; however, there were no significant ($F = 3.10$, $df = 2$, $P = 0.0671$) tree by treatment (infested or clean) interactions.

Acquired Fungal Enzymes. The experiment we set up for testing whether or not a diet of infested or clean poplar wood affected larval growth was inconclusive. Neither diet showed any cellulase activity; possibly due to ineffective homogenizing. Homogenizing of the diet was done manually, which may have been ineffective in releasing cellulases into solution. Larvae from both diets showed no difference in weight gain over the one week period they were allowed to feed. They were allowed to feed for another week, and half of them did not survive. The experiment was terminated due to an inadequate number of larvae to continue.

pH of *Pr. robiniae* gut contents. Results from the thymol blue pH test suggest that the pH of *Pr. robiniae* gut contents is equivalent or exceeds a pH of 10.0 (Fig. 4 and 5).

Discussion

The presence of cellulase enzymes in the gut contents suggest that *Pr. robiniae* larvae acquire these enzymes exogenously. The presence of reduced cellulose in the gut content also suggest an external source of cellulase. Results from our pressed wood assays suggest that fungal infestation of poplar wood prior to *Pr. robiniae* ingestion may be the source of cellulase enzymes and the reduced cellulose. However, our acquired fungal enzyme test proved inconclusive. The pH of *Pr. robiniae* gut content also casts doubt on our findings. All of our tests were performed at a pH of 5.0, optimal for the purchased cellulase. However, the pH of *Pr. robiniae* gut content is > 10.0 . We were unable to purchase commercial cellulase with an optimal pH of anything other than 5.0, so we could not run our assays at the same pH as the gut content of *Pr. robiniae*. If *Pr. robiniae* do produce cellulases endogenously, they would be optimized for the pH of the gut and not pH 5.0, so our assays would not show the true cellulase activity. Our assays also did not test for the presence of zymogen (enzyme precursors). Some enzymes do not become active until they are needed; if cellulases were not activated until they are released into the gut content, our assays would not show activity in the tissue even though zymogens may be present.

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Tables:

Table 1: Results of our cellulase enzyme assay. A “+” indicates one to ten units of cellulase activity. A “-“ equals less than one unit of cellulase activity. One unit of cellulase will liberate 1.0 μ mole of glucose from cellulose in one hr at pH 5.0 at 37°C.

	C _x -Cellulases (Endo- β -1,4-glucanase)	C ₁ -Cellulases (exo- β -1,4- cellbiohydase)	Cellubiases (β -Glucosidase)
Gut Content	+	+	++
Whole Gut tissue	-	-	-
Whole Gut w/contents	+	+	++++

Figures:



Figure 1: A dissected *Pr. robiniae* larvae showing the tissues being assayed (2005, N.T. Kittelson).

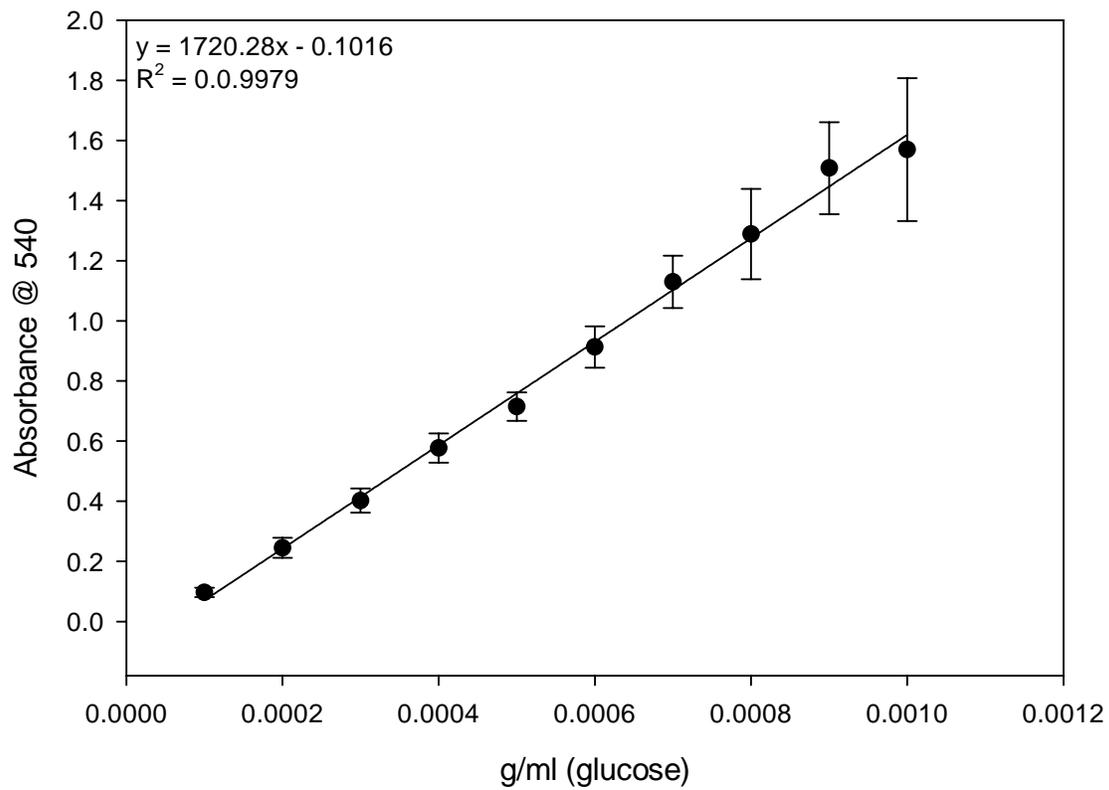


Figure 2: Standard Curve for pressed wood carbohydrate experiment.

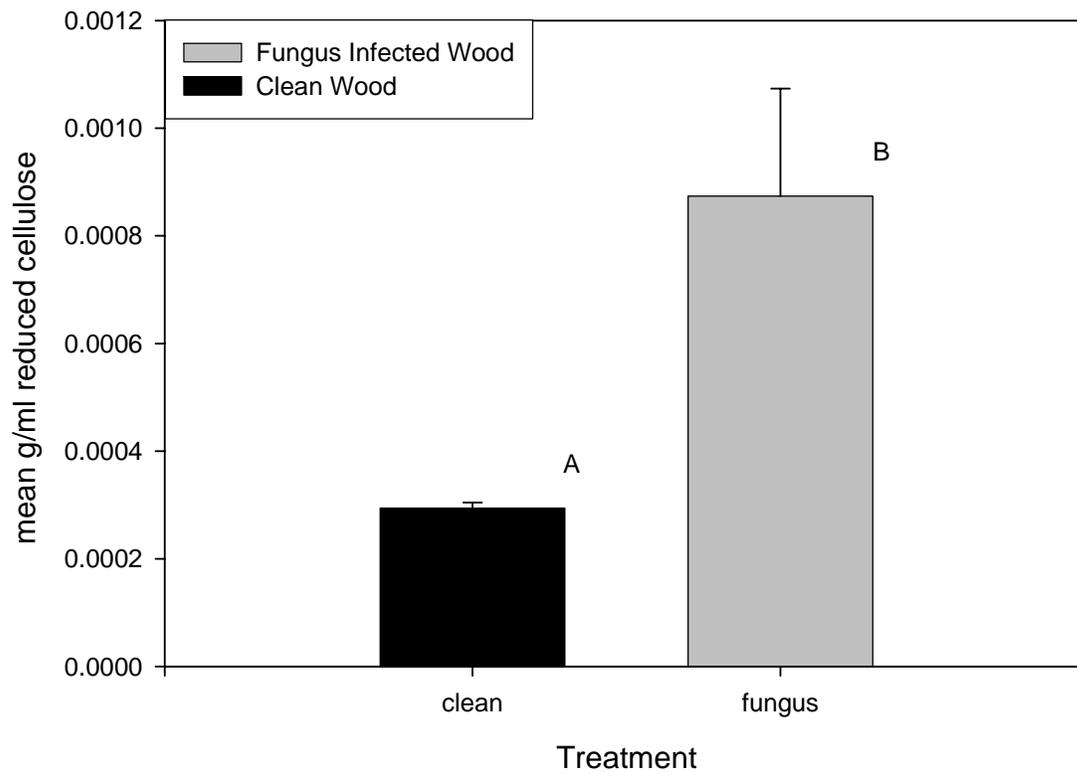


Figure 3: Comparison of carbohydrate found in ‘clean’ and ‘infested’ poplar wood.

Means with the same letters are not significantly different at $P < 0.05$.



Figure 4: Standard curve for the pH sensitive dye, thymol blue (2006, N.T. Kittelson).

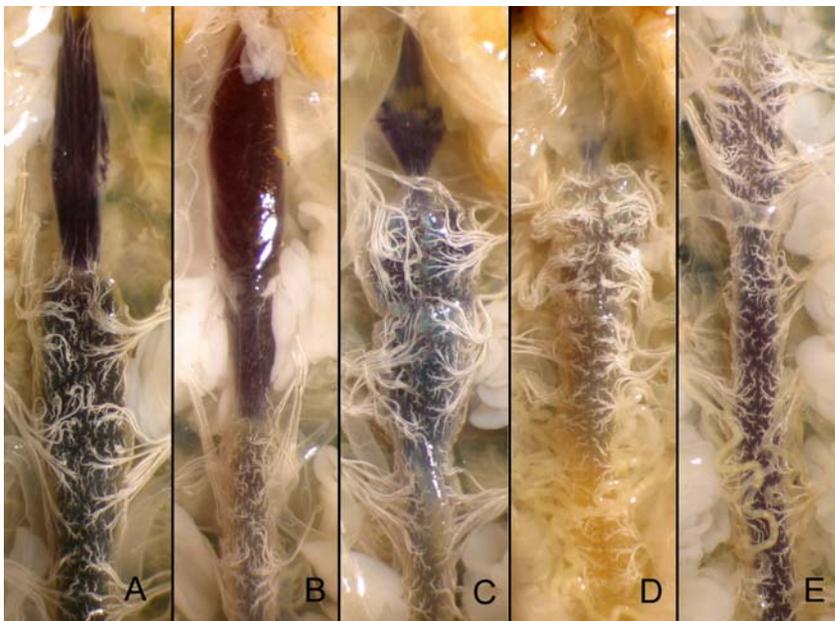


Figure 5: Five (A-E) dissected guts of *Pr. robiniae* larvae fed on a diet of 0.05% thymol blue. The color of the gut indicates that pH is ≥ 10.0 (2006, N.T. Kittelson).