Phoracantha semipunctata (Coleoptera: Cerambycidae), a Serious Pest of Eucalyptus in California: Biology and Laboratory-Rearing Procedures

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ABSTRACT Procedures are described for establishing a laboratory colony of the eucalyptus longhorned borer, Phoracantha semipunctata F., and rearing the adult beetles on a continual basis. Adult beetles reared from naturally infested Eucalyptus logs were caged and provided with oviposition substrates (folded sheets of plastic). Techniques for handling and caring for eggs and neonate larvae are discussed. Larvae were individually transferred into shallow incisions in the bark of fresh logs. Total survivorship from neonate larvae to adult was ~35%. Our procedures yielded an average of 63 adult progeny for every adult female, with a generation time of ~2 mo during the summer. Beetle colonies were protected from pyemotid mites by dusting rearing logs with sulfur and from ants with granular diazinon. An experiment on adult beetle diet showed that the provision of sucrose water greatly increased longevity and fecundity over a distilled water control, but increasing the concentration of sucrose >5% did not significantly improve beetle performance.

KEY WORDS rearing, Eucalyptus, Phoracantha semipunctata

Difficulty in rearing cerambycid beetles has long hindered research on these insects (e.g., Linsley 1959, Payne et al. 1975). Maintaining cerambycids on artificial diet has sometimes proven effective for taxonomic or cytogenetic studies for which relatively small numbers of individuals were needed (e.g., Gardiner 1970, De Viedma et al. 1985). However, rearing cerambycids on artificial diet for behavioral or ecological studies may have three drawbacks: (1) it may be difficult to develop a diet that beetles will eat and that provides all the necessary nutrients; (2) because cerambycids typically have prolonged larval stages (Linsley 1962), rearing on an artificial diet may be very labor intensive because of the need to transfer larvae to fresh media periodically (Linit 1985); (3) artificial diet may influence adult physiology and behavior, resulting in differences between field and laboratory insects (e.g., Stanić et al. 1989). Rearing cerambycids in their natural hosts eliminates these dietary artifacts and can be simpler and more productive than using an artificial diet.

The eucalyptus longhorned borer, Phoracantha semipunctata (F.), is a severe pest of eucalyptus in many countries (Chararas 1969a, Scriven et al. 1986, Hanks et al. 1990). However, adults of both sexes are strongly attracted to stressed Eucalyptus trees and logs of any size as oviposition sites (Chararas 1969b, Drinkwater 1975, Ivory 1977, González-Tirado 1986, Scriven et al. 1986, Mendel 1987, personal observation). Females oviposit under loose bark and in bark fissures in batches of up to 40 eggs. Neonate larvae then penetrate the bark and feed along the cambium.

Heavy infestations of P. semipunctata larvae result in destruction of virtually the entire cambium layer and the rapid death of the tree (Chararas 1969b, Drinkwater 1975, Scriven et al. 1986, personal observation). Fully grown larvae burrow into the sapwood where they construct a pupal chamber, plugging the passage to the log surface with wood chips. During the warm season, the larvae rapidly pupate but will become quiescent over the winter. Overwintering larvae pupate in spring and then emerge as adults, chewing their way out through the pupal plug in late April to mid-May in southern California (personal observation). Emergence is not tightly synchronized, and adult beetles are present continually from spring until late fall. Flight activity of the adult beetles is restricted to warm nights when the temperature at dusk is above ~15°C (Chararas 1969b, Löyttyniemi 1983). In southern...
California, *P. semipunctata* completes two overlapping generations per year (Hanks et al. 1990).

Artificial diets have been developed for *P. semipunctata* (Chararas 1969a, De Viedma et al. 1985), but for the reasons described above were unsuitable for the large-scale, continuous production of adults that we required. We present here information on techniques for rearing *P. semipunctata* on its natural host which are effective in producing large numbers of beetles, and we provide new information on the biology of this important pest.

Materials and Methods

Rearing and Handling Adult Beetles. We obtained logs from several species of *Eucalyptus* that were naturally infested by *P. semipunctata* from campuses of the University of California at Riverside and San Diego. These logs were <50 cm in circumference and were cut into lengths of =0.5 m for ease of handling. On 19 March 1990, we placed these logs upright in screened cages (1.3 m high, 0.65 m deep, 0.55 m wide). Because adult *P. semipunctata* are negatively geotactic, we could collect emerging adult beetles by capturing the cage with a pyramidal screen top terminating in an inverted plastic funnel over which was positioned a 2-liter cardboard container. Adult beetles were collected from cardboard containers daily to reduce injury from fighting.

Cages for rearing adults were kept in a controlled environment chamber (=30 ± 5°C under natural daylight; humidity was not controlled and was near ambient with a mean of =40% RH). Sulfur dust (F.M.C., Philadelphia, PA) was liberally applied to the logs (=20 ml per cage) to inhibit attack by a pyemotid mite that rapidly paralyzed emerging beetles (Hanks et al. 1992). Cages were examined frequently for moribund or dead beetles that might be paralyzed by mites and later become a source of mite infestations.

To examine the temporal pattern of emergence of adult beetles from logs, we recorded the numbers of beetles emerging in five cages on a weekly basis until logs were no longer productive. Each cage contained logs originating from the same location (either University of California Riverside or San Diego) on the same date (during spring 1990).

Adult beetles captured in cage traps were handled by their heavily chitinized antennae with plastic-tipped forceps. This handling technique prevented the beetles from holding onto the forceps and did not appear to damage the antennae. Adult beetles were maintained under laboratory conditions (18–24°C, =40% RH, natural daylight).

Egg Production. To obtain *P. semipunctata* eggs in quantity, adult beetles were housed in glass aquaria (50 cm long, 26 cm wide, 30 cm deep) covered with aluminum window screen. The counter top on which cages were kept was lightly dusted with talc (Humco Laboratory, Texarkana, TX) to inhibit the movement of pyemotid mites (Hanks et al. 1992). As many as 20 beetles were kept in each aquarium. Each cage was provided with 30% sucrose solution in 8-ml vials with a 4-cm-long number 2 cotton roll (Patterson Dental, South Edina, MN) inserted halfway into the vial. We provided one of these feeders for every three beetles. A small amount of honeybee pollen (0.5 g per cage) was also provided.

Oviposition substrates consisted of sheets of 6-mil polyethylene sheeting cut into strips (10 cm long, 6 cm wide) that were stacked five strips thick, folded once lengthwise, and stapled. These folded sheets were laid on the top screen of the cage with their loose-leaf sides down, providing an abundance of folds into which a female could insert her ovipositor up through the screen. These oviposition substrates worked best when weighted down slightly to keep them in place. We replaced the oviposition substrates daily so that all eggs in each substrate were of the same age, thereby synchronizing hatching.

Handling Eggs and Rearing Larvae. To determine survivorship of eggs to hatching, 200 freshly laid eggs were monitored until they hatched under ambient laboratory conditions (=20°C and =40% RH) in March 1992.

Neonate larvae were manually transferred to logs to control larval density and to provide cohorts of the same age. Logs for rearing *P. semipunctata* larvae were cut into lengths of 0.5 m from freshly cut *Eucalyptus camaldulensis* Dehnhardt trees having an average trunk basal circumference of 40 cm. *P. semipunctata* larvae require logs that are fresh to complete development (Chararas 1969b, Mendel 1985). To preserve the moisture content of logs, we waxed the ends of the logs by dipping them in paraffin wax (Bromar, Newport Beach, CA) heated until just smoking. Allowing the logs to dry out for 24 h before waxing reduces bark turgidity which can inhibit the movement of neonate larvae in the bark and prevent their establishment (Hanks et al. 1991a). Waxied *Eucalyptus* logs stored at 5°C remained viable hosts for the larvae for as long as 2 mo.

Larvae were introduced into logs by cutting a slit in the bark a few millimeters wide and deep enough to reach the cambium, then transferring the larvae into this slit with a damp fine-tipped paintbrush (size 00000). Once in place, the larvae were protected by a cover of paper (such as paper toweling) taped over the slit, and the logs were left in place for 24 h to prevent the larvae from being dislodged before they bored into the cambium. Because survivorship of larvae decreases with their density in the log (Hanks et al. 1991b), the number of larvae introduced into the logs should be such that the resulting density is low (=50 mature larvae per square meter of bark.
represents an optimal density for *E. camaldulensis* [L.M.H., personal observation]). Using an initial density only slightly in excess of this density ensures that the maximum density will be reached but without significant mortality from competition. We infested nine logs of *E. camaldulensis* (surface area 0.24 ± 0.02 m² with 20 larvae (average initial density of 83 larvae per square meter of bark) on 16 August 1990 and wrapped each log in organdy to capture emerging adult beetles. Logs were maintained in a temperature-controlled rearing facility (=30 ± 5°C, 40% RH, natural daylight) until emergence ceased. It was crucial that ants be excluded from rearing facilities because the bark of heavily infested logs often cracks, exposing the defenseless larvae or pupae to predation. We sprinkled granular diazinon (Ortho Consumer Products, San Ramon, CA) lightly around the legs of the log shelves to discourage ant attack.

After adult emergence ceased, we removed the bark of each study log to measure the following three variables: (1) the number of first instars reaching the cambium, determined by counting larval galleries; (2) the number of larvae reaching the prepupal stage, determined by counting holes where mature larvae had entered the sapwood to pupate; and (3) the number of pupae reaching adulthood, determined by counting opened emergence holes. These data allowed us to calculate survivorship of larvae during colonization, larvae to pupal survivorship, pupal survivorship, and total survivorship from first instar to adult. Adult Feeding Experiment. Adult *P. semipunctata* have been maintained in the laboratory with varying results using a variety of diets including honey (Duffy 1963), simple sugars or simple sugars plus leaf extracts (Chararas et al. 1971), and malt extract (Ivory 1977). We have observed the adult beetles feeding on *Eucalyptus* flowers in the field and attempted to develop a diet for adults that would provide similar nutrients. The experiment was conducted beginning 18 June 1991 to determine the concentrations of sucrose with or without pollen that would maximize the reproductive rate. Individual mated pairs of newly emerged adult beetles were presented with eight diets: 5, 10, or 30% sucrose in distilled water, honeybee pollen (0.1 g; primarily pollen from asteraceous flowers) (Clark’s Nutrition, Riverside, CA) and distilled water, pollen (0.1 g) and 10% sucrose water (distilled), pollen (0.1 g) and 30% sucrose water, 10% honey water (distilled), and distilled water alone. The amount of pollen used in the pollen treatments (0.1 g) exceeded the amount that beetles would consume before replacement. Beetle pairs (male and female) were caged in cylinders made from aluminum window screen that were 12 cm high and 12 cm in diameter with 14-cm-diameter plastic petri dishes on the top and bottom. A 12.5-cm-diameter circle of no. 1 filter paper (Whatman International, Maidstone, England) was placed in the bottom of the cage. Distilled water and sucrose or honey solutions were presented in glass vials (as described above). Two vials were put into each cage, laid on their sides in a smaller petri dish (8.5 cm diameter) to keep them from rolling onto beetles. Both water solutions and pollen were changed every other day.

**Phoracantha semipunctata** mated readily and repeatedly during the active period (at night), and all eggs produced were fertile. In the screen cages, females oviposited readily between the filter paper and the smaller petri dish, making it easy to determine fecundity. At least five pairs of beetles were used in each treatment, and longevity and fecundity were recorded. Analysis of variance was used to examine the effects of diet on longevity and fecundity (SAS Institute 1988), and treatment means were compared using mean separation tests (Waller-Duncan *t* tests) (SAS Institute 1988). No voucher specimens from this study were retained.

Results and Discussion

Rearing and Handling Adult Beetles. The funnel trap at the top of the cages was effective in capturing adult beetles that emerged from logs. Adult *P. semipunctata* began emerging from the naturally infested logs beginning on 30 March 1990, 5 wk after caging. In total, 975 adults emerged over a 16-wk period (Fig. 1). Although there were clear peaks in emergence between weeks 11–15, emergence was not tightly synchronized and beetles emerged continually over a 16-wk period. These findings support the observation that adults in the field emerge from logs for extended periods with no distinct generations (Chararas 1969a; Loyttyniemi 1983; Mendel 1985). Emergence of adult *P. semipunctata* from logs containing prepupae can be more closely synchronized if the logs are cold-treated (−5 to +10°C for 30 d) before they are transferred to warm (25°C) rearing conditions (Hanks et al. 1991).

Adult *P. semipunctata* in the laboratory cages were quiescent during the day but walked rapidly around the cage from shortly after dusk until dawn. Because *P. semipunctata* males will fight among themselves for possession of females, the sex ratio in the laboratory cages was adjusted to strongly favor females. A ratio of one male to three females was effective in reducing conflicts and injuries while maintaining high fecundity.

**Egg Production.** Female *P. semipunctata* laid eggs in the oviposition substrates in batches of up to 30 eggs. Eggs adhered to the plastic sheets and so could be easily moved to glass jars. Eggs within a batch hatched synchronously within one day. *P. semipunctata* eggs could be stored
for as long as 7 d at 16°C in covered petri dishes containing a moist Kimwipe (Kimberly-Clark, Roswell, GA) to maintain high humidity.

**Handling Eggs and Rearing Larvae.** From a batch of 200 *P. semipunctata* eggs, 196 (98%) successfully hatched. Larvae could be easily collected by keeping oviposition substrates containing eggs in a 1-liter glass jar. Larvae hatched from eggs and fell to the bottom of the jars in =5 d at 20°C. In the absence of food and under laboratory conditions, first instars remained viable for only =3 d. However, the longevity of neonates may be prolonged to 7 d when stored at 16°C.

There is a strong host plant species effect on the performance of *P. semipunctata* larvae (Powell 1978, Löyttyniemi 1983). Therefore, it is critical that the host log species be considered in rearing this cerambycid. We have found *E. camaldulensis* to be among the highest quality hosts.

Infesting logs with *P. semipunctata* larvae by hand makes it possible to control larval density in logs. By using lower densities, we could maximize productivity by limiting competition among larvae, thus decreasing development time and increasing both body size and fecundity. Larvae required =40 d to complete development to the prepupal stage under laboratory conditions. When 20 larvae were introduced by hand into each of nine *E. camaldulensis* logs, an average of 17.1 ± 3.7 (mean ± SEM) succeeded in colonizing the logs, for a survivorship of 85.5%.

The average number of these larvae reaching maturity (i.e., constructing a pupal cell) was 12.6 ± 4.0, or 73.4% survivorship.

The number of pupating beetles emerging as adults was 7.0 ± 2.1 adults per log, or 55.5% survivorship. Nonemerging beetles died in prepupal, pupal, and teneral adult stages for unknown reasons. This level of mortality in the pupal chambers is similar to that observed in the field (L.M.H., personal observation). On average, the survivorship of the 20 larvae per log from first instar to adulthood was 35%, even in the absence of predators and parasites.

With an average fecundity of =180 eggs per female (see below), we were able to infest nine logs with 20 larvae each using progeny of a single adult female. Given a survivorship of first instars to adulthood of 35%, our culturing techniques allowed us to produce an average of 63 adult *P. semipunctata* for every female from the previous generation.

**Adult Feeding Experiment.** Longevity among beetles provided with only distilled water was shorter than for beetles provided with 10% honey water (*F* = 3.26; df = 7, 78; *P* = 0.0043) (Fig. 2A). A diet of only sucrose solutions (5, 10, 30%) resulted in survival times that were comparable with those of the honey water diet. Beetles provided with only pollen had a mean survival time not significantly different from those fed only distilled water, suggesting that they were obtaining insufficient nutrition from pollen. A combination of pollen and sucrose solution resulted in longevity similar to that of beetles fed on honey or sucrose solutions.

These results suggest that the addition of sucrose greatly improves longevity, but there is no advantage in increasing the concentration of su-
Fig. 2. Longevity (A) and fecundity (B) of *P. semipunctata* on eight diet treatments. Means ± SEM marked with different letters are significantly different (*P* < 0.05; Waller–Duncan *t* tests).
sucrose above 5%; that the addition of pollen to a sucrose diet did not improve longevity over a sucrose solution alone. Microscopic examination of frass produced by study beetles showed partially digested pollen, confirming that beetles did eat the honeybee pollen. We have also found partially digested *Eucalyptus* pollen in the frass of freshly caught feral adult *P. semipunctata*, indicating that pollen is a natural food source. The results described above indicate that honeybee pollen is not of significant nutritional value to the beetles in the laboratory, because pollen-fed beetles had longevities equal to those maintained on distilled water alone. However, pollen may provide amino acids that are used in flight (Candy 1989).

The 30% sucrose, 10 and 30% sucrose + pollen, and 10% honey water treatments all resulted in significantly higher fecundity than did the distilled water control (*F* = 2.55; *df* = 7, 37; *P* = 0.03) (Fig. 2B). Although fecundity in the 5% sucrose treatment (116 ± 45.5 eggs per female [mean ± SEM]) was >16 times that of the distilled water control (7.7 ± 7.5 eggs per female) treatment, this difference was not statistically significant because of the great amount of variation in fecundity within treatments. However, when the sucrose data (5, 10, and 30% sucrose) were combined into one “sucrose” treatment, and the pollen plus sucrose data (10 and 30% sucrose plus pollen) were combined into a single “sucrose plus pollen” treatment, there were no significant differences between the honey water, sucrose, or sucrose plus pollen treatments (respective means ± SEM, 188.7 ± 46, 123 ± 22, and 186 ± 31 eggs per female), but all three were significantly higher than the distilled water treatment (7.7 ± 7.5 eggs per female; *F* = 4.66; *df* = 4, 40; *P* = 0.0035). These data strongly indicate that fecundity can be improved nearly 20-fold over a distilled water diet when sucrose or honey are provided. As with longevity, fecundity was not improved by increasing the concentration of sucrose above 5%. We have found honey water to be less practical than sucrose for rearing beetles because honey water ferments after 24 h whereas a sucrose solution remains potable to the beetles for as long as 3 d.

Using these methods, we have maintained a large colony of *P. semipunctata* in the laboratory for >3 yr. Although the production of adult beetles can be somewhat cyclical because of the long emergence period of the larvae, our colony has yielded as many as 25–50 adult beetles per day for a total of >10,000 beetles in a single year with a sex ratio of 57% female.

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