

TECHNIQUES FOR REARING THE WOOD BORERS
PRIONOXYSTUS ROBINIAE (LEPIDOPTERA : COSSIDAE)
AND *PARANTHRENE DOLLII* (LEPIDOPTERA : SESIIDAE)

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Studies of lepidopteran wood borers are frequently limited by lack of effective rearing methods to produce adequate numbers of these insects. Although the carpenter-worm, *Prionoxystus robiniae* (Peck), has been reared in the laboratory, a number of problems persist, including space requirements, mating procedures, diet composition and microbial contamination. Rearing space to accomodate larval rearing containers has been a limiting factor in artifical culture (Rivas & Buchanan 1958, Solomon & Abrahamson 1976). A number of diets, including the one used by Solomon (1966), incorporate sawdust. Fungal and bacterial contaminants frequently occur limiting the utility of other diets (Leppla et al. 1975). Adoption of a meridic diet containing microbial inhibitors with commercially available ingredients may be more advantageous. In addition, a mating technique, essential to maintaining any laboratory culture, has not been reported.

We developed a system for rearing *P. robiniae* for laboratory bioassays by adopting a commercially-available meridic diet to reduce contamination in the culture containers, reducing space requirements for rearing operations and incorporating a successful mating technique. In addition, similar techniques were evaluated for rearing another wood boring species, *Paranthrene dollii* (Neumoegen).

Our *P. robiniae* colony was initiated in May, 1985, using 6,000 eggs obtained from forest-reared adults provided by J.D. Solomon (U.S. Forest Service, Stoneville, Miss.). The *P. dollii* colony was initiated with 250 eggs obtained from adults emerged from field-collected cuttings of *Populus deltoides* (Bartr.) also provided by J.D. Solomon. Eggs of both species were incubated at room temperature ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$) in petri dishes (60 X 15 mm) lined with filter paper. Petri dishes containing eggs were held in covered plastic containers (26 X 9 X 9 cm) lined with moistened paper toweling to maintain a constantly humid environment.

Larvae of both species were reared on a modification of the lepidoperan diet described by Yearian et al. (1966). Changes in the ingredient amounts were: 12 g methyl parahydrobenzoate, 70 g agar, and 2900 ml deionized water. The vitamin mixture of the original diet was replaced with 50 g of Vanderzant vitamin mixture. *P. robiniae* neonates were placed two per 30-ml polystyrene diet cup containing 10 ml of diet and maintained at room temperature with a photoperiod of 16:8 (L:D). Approximately one month later, larvae were individually transferred as 3rd- and 4th- instars to petri dishes (100 X 15 mm) containing diet. These rearing dishes had been prepared by pouring hot diet to a depth of 3-5 mm in the dishes, allowing the diet to cool and solidify, and removing half of the diet to an empty dish. Dishes containing larvae were held in covered plastic containers (26 X 19 X 9 mm). Larvae were transferred to fresh diet at monthly intervals for three months and ca. once every two months thereafter.

At 13 weeks one half of the colony was placed in total darkness at 7°C . These were returned to room temperature and held in total darkness 9 to 1 weeks later. The remainder were maintained at room temperature on a 16:14 (L:D) regimen throughout the remainder of the larval period (13-28 weeks).

Pupae were sexed and then transferred to 0.5 liter paper Fonda^R cartons (up to 10/carton) containing a 2-cm depth of moistened vermiculite. The Fonda^R carton lids

were modified by replacing the paper center with a 18-mesh wire screen circle (12-cm diameter) shaped into a dome. The individual emergence chambers were loosely covered with Reynolds 915 plastic wrap to maintain a humid environment.

Males were kept in a room separate from the females to prevent the adult moths from becoming acclimated to the female pheromone. Other precautions, were routinely practiced, such as autoclaving emergence/mating chambers after possible contamination with pheromone and holding females near an exhaust vent.

Upon emergence, males were taken to the room containing a 'calling' female. Receptive males responded with the characteristic "crawl-dance-flight" described by Solomon (1973). Once this occurred, the screen domes of the emergence chambers were placed together allowing male/female contact. If copulation was not observed within 15 minutes, the male was removed and another male, if available, was tested. After copulation, which averaged ca. 1 hour, the moths separated. Females were then placed in 0.5-liter Fonda^R cartons lined with crumpled paper toweling to serve as an oviposition substrate and capped with a 100 X 15 mm petri dish lid.

P. dollii larvae and pupae were managed and maintained in a manner similar to that described for *P. robiniae*. However, larvae were not subjected to a cold treatment but were maintained at room temperature on a 16:14 (L:D) regime throughout their development. The emergence chambers were further modified by attaching a tripod base constructed by taping three cardboard lids (Fisher Brand^R lids for 210 ml specimen containers) together so that the pull tabs formed a tripod. This stage was covered with paper toweling and placed atop moistened vermiculite. This arrangement provided a dry arena for the pupae while maintaining sufficient humidity to facilitate eclosion.

Sexes remained in separate rooms until initiation of copulation. Mating chambers were the same as described for *P. robiniae*. Mating proficiency of *P. dollii* was increased by increasing the number of males (up to 4) per female at mating. Both sexes were sometimes caged together for up to six hours before successful copulation occurred. After mating, females were placed in ovipositional chambers consisting of petri dishes (150 X 25 mm) with the lid lined with filter paper (15-cm diameter). Oviposition was stimulated by placing a *P. deltiodes* leaf under the filter paper.

Microbial contamination of diet containers was ca. 7% per generation and was mainly *Aspergillus* spp. Contaminated cups or dishes were routinely discarded to prevent further contamination.

The *P. robiniae* egg incubation period ranged from 14-19 days at room temperature. Percentage hatch was 96% for the first generation and 91% for the second. Establishment of neonate *P. robiniae* was greater than 95%. Percentage overall survivorship of two consecutive carpenterworm generations reared on the artificial diet was 11% for the first generation and 28% for the second. The maturation data from first generation (1985-1986) initiated with 800 neonates showed 55% matured to late instars (> 7th instar) 18% pupated and of those that pupated 62% emerged. The second generation (1986-1987), initiated with 650 neonates, had 67% mature, 45% pupate and 68% of the number that pupated emerge. In 1986, separate records were kept on the refrigerated and non-refrigerated groups. As reported by Leppla (1985), the larvae developed at the same rate regardless of photoperiod or temperature regime. Developmental time from hatch to adult eclosion was approximately 300 days. The majority of the mortality recorded during the larval period occurred after 15 weeks and was attributed to desiccation. The pupal period lasted from 14-20 days. Male and female adult longevity was 4-5 days. However, male longevity was increased by placing them at a lower temperature as described by Solomon (1977). The sex ratio was approximately 1:1 for both years and emergence began ca. nine months after neonate establishment. Moths emerged for the next 150 days with sufficient synchrony of male and female eclosion to permit continuation of the colony. Mating success, as measured by the percentage of emerged

females mated, was 44% and 61% for 1986 and 1987, respectively. Female egg production was 318 (SD ± 25) eggs/female in 1986 and 207 (SD ± 37) eggs/female for 1987. Eighty percent of the females, dissected after oviposition, had laid their entire egg complement.

The egg incubation period for *P. dolli* was 14-17 days and 85% hatched. Establishment of neonate *P. dolli* was approximately 50%. The resulting generation time was 150 days. Of the larvae established, 73% pupated from which 56% emergence was recorded. Overall survival from larval establishment to adult eclosion was 44%. The sex ratio upon emergence was 1:1 and was synchronous. Percentage of emerged females mated was 27% and fecundity averaged 207 (SD ± 18) eggs/female.

Observations made during these studies include the occurrence of white, rather than the usual red, *P. robiniae* larvae. Those larvae (n=38) that lacked the normal pigmentation were identified and segregated. The adults produced by these larvae were indistinguishable from those produced by normal larvae. However, two white male-white female matings were made and the larvae produced were only white. Emerged *P. dollii* adults exhibited two distinct color forms. Approximately 60% were dark brown while the remainder were cinnamon colored. The number of yellow abdominal cross bands ranged from zero to six.

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