ACTIVITY RHYTHMS, INFLUENCE OF HOST PLANT ON MATING AND OVIPOSITION, AND REARING OF THE SOUTHERN PINE CONEWORM (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

The southern pine coneworm, Dioryctria amatella (Hulst), (Lepidoptera: Pyralidae) is nocturnal and reproductive behavior occurs only during the last 5 h of scotophase (12L:12D photoperiod). Mating does not begin until moths are 2 days old and mating and oviposition are dependent on the presence of the host plant. Satisfactory rearing required surface sterilization of eggs with sodium hypochlorite, autoclaving the dietary wheat germ, inclusion of zoxylic acid in the larval rearing medium, and a larval rearing density of no more than 2 per 30 ml container. The described methods have been used to rear more than 40 successive generations of the insect on the WGCS medium.

RESUMEN

El gusano de piñón, Dioryctria amatella (Hulst), (Lepidoptera: Pyralidae) es nocturno y su funcionamiento reproductivo toma lugar solamente durante las últimas 5 horas del período de oscuridad (12L:12D fotoperíodo). El apareamiento no comienza hasta que las polillas tengan 2
Larvae of the southern pine coneworm, *Diorystria amatella* (Hulst) damage southern pines by feeding on male and female strobili, on vegetative tips, on strobili infected with the rust *Cronartium stroblinum* (Arth.) Hedge and Hahn, and on fusiform rust galls caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Cumm.) Burds., et Snow (Ebel 1963, 1965, Neunzig et al. 1964, Coulson and Franklin 1970, Ebel et al. 1975). Larvae pass through 5 instars (Fatzinger 1970a) and require from 3 to 5 weeks to develop to the pupal stage when reared at ca. 24°C on pine cones (Ebel 1965). The species is multivoltine and may have from 1 to 4 generations per year in north Florida (Ebel 1965, Merkel and Fatzinger 1971).

Previous attempts to establish laboratory colonies were only partially successful because of insufficient egg production between generations. Ebel (1959) reared *D. amatella* from the egg to adult stage with procedures developed for *Diorystria eboli* Mutuura and Monroe (formerly *D. abietella* (Denis & Schiffermüller)), on waxed conelets of slash pine, *Pinus elliottii* Engelm var. *elliottii*. DeBarr (1968) reared larvae of *D. amatella* on the artificial medium developed by Fatzinger (1968) for *D. eboli* but could not obtain enough eggs for continuous culture. Merkel and Fatzinger (1966) reared *D. amatella* on slash pine cones and obtained an average of only 30 eggs per female deposited on small fusiform rust galls cut from slash pine branches. Ebel (1965) observed that only 16% of newly emerged females held in mating cages deposited viable eggs during the first 2 days whereas 43% contributed by day 5. DeBarr (1968), however, found that only 10-20% of the females mated in the laboratory whereas about 50% did so in an outdoor insectary.

The study reported here was conducted to optimize the rearing of *D. amatella* by determining its temporal patterns of reproductive behavior, requirements for host plant material to stimulate mating and oviposition, tolerance of increased larval densities, reaction to surface sterilization of eggs with sodium hypochlorite or peracetic acid, and acceptance of sterilized dietary ingredients.

**MATERIALS AND METHODS**

A laboratory colony of *D. amatella* was initiated in 1970 with larvae collected from fusiform rust galls of slash pine and eggs from moths that emerged from slash pine cones. The larvae were reared on the WGCS medium developed for *D. eboli*; constituents include wheat germ, casein, 5 sugars present in slash pine cones, salts, vitamins, 2 mold inhibitors, agar, and water (Fatzinger 1970b). In a slight modification, the medium was thickened by decreasing the water content 25% and the nonessential stach-
yose was eliminated. The hot (35°C) medium was dispensed into either 30 ml plastic condiment cups or sterilized glass test tubes and allowed to cool (gell) prior to initiating rearings of first-stage larvae. The cups were filled to a depth of 1 cm with medium. The test tubes were filled to a depth of 4 cm and gelled at a 30° angle to provide a thickness-dependent moisture gradient which enabled larvae to select optimum conditions for feeding. After the larvae were transferred, the tubes were stoppered with sterile, nonabsorbent cotton and the cups were capped with plastic, snap-on lids. The resulting pupae were separated by sex (Fatzinger 1968) and held on moist paper toweling in wire screen cages (30 x 30 x 30 cm) until moths emerged. The environment for all rearing and experimentation was 27°C, ca. 65% RH, and 12L:12D photoperiod (0600 to 1800 photophase).

Locomotor activity, female calling, and mating behavior were recorded automatically by time-lapse photography (Fatzinger 1973). Moths were photographed through the glass doors of clear plastic cages at 30-min intervals for 7 days.

Host material consisted of cross sections (ca. 6 mm thick x 4 cm dia.) cut from a fusiform rust branch gall. Initially the behavior patterns of 26 ♀ moths and 9 pairs of ♀ moths that emerged from field-infested slash pine cones were observed without host plant materials. Behavioral effects of host plant material were observed with moths obtained from the first 15 successive generations reared. These included 9 ♀ observed without galls and 18 ♀, 20 ♀, and 14 pairs observed with galls. The general patterns of behavior were obtained by averaging the observations of all moths regardless of their age. The times of the occurrence of behavioral events are reported as means in hours and minutes ± angular deviations in tenths of hours (P = 0.01). These means were obtained by the methods described by Bateschlet (1965) for circular normal distributions.

Over a 3-year period, 26 colonies of D. amatella were initiated to assess the effect of larval rearing density on colony survival and duration of larval stage. Tests included 11 colonies or 14,045 larvae reared 1 per container (899 in test tubes and 13,146 in cups), 4 colonies or 764 larvae reared 2 per container (140 larvae in test tubes and 624 larvae in cups), and 11 colonies or 28,482 larvae reared 3 per container (4,776 in test tubes and 23,706 larvae in cups).

Eggs and constituents of the WGCS medium were individually plated on sterilized malt agar and nutrient agar media in plastic petri dishes to determine the sources of microbial contaminants. The dishes were held at 22°C and were observed for 2 weeks at 2-day intervals for the growth of microorganisms.

The efficacies of peracetic acid and sodium hypochlorite for sterilizing the egg surfaces were compared. Eggs deposited on cheesecloth were washed for 1 min in a detergent (0.1% sodium alkylarylsulfonate), submerged for 5 min in either 0.1% peracetic acid or 0.5% sodium hypochlorite solutions, and rinsed twice in sterile water. Individual eggs were transferred to plates of both culture media (30 plates of culture media containing 3 eggs each per treatment).

The egg sterilization methods also were evaluated by rearing 100 larvae that hatched from eggs treated with peracetic acid and 100 larvae from eggs treated with sodium hypochlorite. These larvae were reared on the WGCS
medium and the percentage of contaminated rearing containers, the average percentage of surface contamination, and the survival percentage to the pupal stage were compared between the treatments. Controls included 100 larvae hatched from untreated eggs and 100 rearing containers that held WGCS medium but no larvae. The brush used to transfer larvae to rearing containers was sterilized with Amphyl® disinfectant after every third transfer.

Samples of the constituents of the WGCS medium were plated on 10 replicate dishes of each culture medium to evaluate this source of contamination. To reduce contamination in the WGCS medium, its ingredients were treated in the following ways: (1) wheat germ was sterilized by autoclaving at 15 psi for 30 min prior to mixing the medium, termed AUTOWG; (2) 0.25% sorbic acid was added to AUTOWG as an additional mold inhibitor, termed AUTOWG+S; (3) the WGCS medium was autoclaved at 15 psi for 30 min, termed AUTOWGCS; (4) the wheat germ was sterilized over propylene oxide for 24 h prior to mixing the medium, termed FUMWG; (5) the wheat germ was sterilized with dry heat for 4 h at 180° C prior to mixing medium, termed DRYWG; and (6) the standard WGCS medium, termed CHECK.

The initial generation of D. amatella reared on each experimental medium was started with surface sterilized eggs collected from generations 27 through 34 of the laboratory colony. First-stage larvae were reared individually to the pupal stage in test tubes containing the experimental media. The number of days until first visual evidence of contaminated media, number of days to larval death, and duration of the larval stage were recorded.

Darkened pupae from each medium were held in individual 25 x 95 mm vials that contained a piece of 1 x 6 cm screen and were fitted with a cork stopper. The sex and weight of the pupae, and the duration of the pupal stage were recorded for each individual. Samples of moths that emerged on the same day from each experimental medium were transferred to wire screen cages (30 x 30 x 30 cm) for mating and egg deposition. The moths were provided water from a sponge held in an uncovered petri dish. The number of cages observed for each medium depended on the timing of moth emergence and the numbers of emerging moths. Egg deposition was recorded in an average of 4 cages (range 1 to 6) per replicate; the grand average was 14 cages (range 3 to 34) per treatment. The average number of moths per cage was 4.8 (range 2 to 10) and the average sex factor (ratio of females to total) was 0.4 (range 0.2 to 0.6) per cage. Each cage contained 2 cross sections (ca. 1.5 cm thick x 4 cm dia.) cut from fusiform rust galls of slash pine because host plant material was essential for mating and oviposition. Each cross section of gall was wrapped in 2 layers of cheesecloth upon which moths deposited eggs. The galls were replaced every 2 days with freshly cut cross sections and wrappers. The cheesecloth wrappers facilitated removal of eggs from the galls and supported the eggs during surface sterilization. Numbers of eggs deposited on the cheesecloths and numbers of larvae hatching from each batch of eggs were determined daily. The generation time was calculated by summing the average duration of development for female insects from egg to adult and the average preoviposition period.

Differences among treatment means were tested for statistical sig-
nificance by analysis of variance and Duncan's multiple range test, and are reported as means ± standard errors. Prior to these analyses, data collected as percentages were transformed to arc sin \( \sqrt{\text{proportion}} \), but only the percentages are listed in the tables and results.

**RESULTS AND DISCUSSION**

**BEHAVIOR OF MOTHS AND EFFECTS OF HOST PLANT MATERIAL**

A t-test for paired observations revealed no significant difference (\( P > 0.01 \)) between diel periodicities of locomotor activity of male and female moths. Locomotor activity began at 1825 ± 0.5 (N = 165) and ended at 0532 ± 1.7, i.e., it began about 25 min after dark and continued until ca. 28 min prior to the onset of photophase. During each photophase, moths generally remained motionless unless disturbed. The pattern was similar to that reported for *D. ebeli* (Fatzinger 1973) and the presence or absence of a fusiform rust gall had no effect. However, *D. amatella* calling, mating, and oviposition did not occur in cages without fusiform rust galls (26 ♀ moths and 9 pairs of moths). In contrast, *D. ebeli* readily mated and oviposited in laboratory cages without host plant material (Ebel 1959, Merkel and Fatzinger 1966, Fatzinger 1970b).

When the females were 2 days old, calling behavior began ca. 8 h after dark and ended ca. 2.5 h later. Two- to 5-day-old virgin moths began to mate ca. 9 h after dark and ceased to mate ca. an hour later (Fig. 1). These ob-

![Graph showing periodicity of locomotor, calling, and mating activity of *Dioryctria amatella*.](image-url)
servations agree with the delayed mating reported by Ebel (1965). Multiple mating at intervals of 1 to 5 days was observed in both males and females; 3 ♀ and 2 ♂ mated twice and one pair mated 3 times.

**Effect of Larval Rearing Density**

During this study the same quantity of food was placed in each rearing container regardless of the number of larvae reared per container. The average numbers of pupae recovered per container from rearing at different larval densities were 0.58 ± 0.04 pupae at 1 larva/container, 1.25 ± 0.14 pupae at 2 larvae/container, and 0.91 ± 0.10 pupae at 3 larvae/container. Survival of pupae was the same at all densities, averaging 88.1 ± 1.6%, and the differences were due to larval mortality. Larval mortality at the highest density was significantly greater than at the 2 lower densities (P < 0.01). Several partially eaten pupae were found, but cannibalism was rare.

Larval density did not significantly (P > 0.01) influence the time required to rear larvae from the first stage through pupation. The average durations were 30.8 ± 1.0, 26.1 ± 1.8, and 28.1 ± 0.5 days for larvae reared 1, 2, or 3 per container, respectively.

Of the 9,494 rearing containers containing 3 larvae per container, ca. 5% were heavily contaminated (95% of the medium surface was contaminated). Only ca. 1% of the rearing containers with 1-2 larvae per container became heavily contaminated. The increased incidence of excessive contamination for colonies reared with 3 larvae per container was probably due to the greater chance of transferring larvae hatched from imperfectly disinfected eggs to a given container.

Dead larvae were found more frequently in excessively contaminated rearing containers. Prior to death, many of these larvae remained in the first or second stage while larvae in other containers progressed to the fourth or fifth larval stage. The physical barrier formed by microorganisms on the surface of the medium apparently caused the larvae to die from starvation (Fatzinger 1970b, Bucher and Bracken 1976).

Some larvae that hatched from eggs oviposited in the fall and winter remained in the first stage for as long as 66 days before dying (Fig. 2). They readily crawled about if disturbed but remained in the first stage long after the remainder of the colony pupated. In North Carolina, *D. amatella* overwinter primarily as early-stage larvae (Nounzig et al. 1984). In laboratory and field populations of *D. amatella* from north Florida, Ebel (1965) found early-stage larvae that ceased active feeding in the fall and winter, and either wandered about or remained inactive among cone scales or bark crevices. The inactive first-stage larvae observed during rearings may have been exhibiting the overwintering behavior of wild populations and they may have died from starvation because the medium deteriorated.

**Surface Sterilization of Eggs**

Eggs collected from the rearing cages were contaminated with both bacteria and fungi. Significantly fewer eggs were contaminated (P < 0.01) after treatment with peracetic acid (33.7%) or sodium hypochlorite (53.7%), but treatment with peracetic acid was superior. However, equal production of pupae was obtained from eggs surface-sterilized by either method (Table
Fig. 2. Number of days Dioryctria amatella larvae lived after hatching during different months and life spans of those that died after being placed on artificial media.

1) and that production was higher than that from untreated eggs. Contamination of rearing containers held without larvae was minimal.

**DECONTAMINATION OF THE LARVAL REARING MEDIUM**

Wheat germ and casein were the primary sources of contaminants in the WGCS medium. All the plates containing wheat germ showed visible growth of microorganisms but only 25% of the plates containing casein showed

**TABLE 1. EFFECT OF SURFACE STERILIZATION OF EGGS ON CONTAMINATION OF REARING CONTAINERS AND SURVIVAL OF Dioryctria amatella REARED ON THE WGCS MEDIUM.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contaminated rearing containers*</th>
<th>Average surface area of medium contaminated**</th>
<th>Survival to pupal stage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peracetic acid</td>
<td>39</td>
<td>13.2 a</td>
<td>80</td>
</tr>
<tr>
<td>Sodium hypoehlorite</td>
<td>55</td>
<td>19.6 a</td>
<td>82</td>
</tr>
<tr>
<td>Non-sterilized eggs</td>
<td>84</td>
<td>41.5 b</td>
<td>52</td>
</tr>
<tr>
<td>WGCS medium only</td>
<td>22</td>
<td>0.8 c</td>
<td>—</td>
</tr>
</tbody>
</table>

* A X² test for independence showed significant differences among treatments at the 1% probability level.
** Visually estimated to the nearest percent in contaminated rearing containers. Any 2 means followed by the same letter are not significantly different at the 1% probability level.
contaminants to be present. Therefore, the 5 modifications of the WGCS medium were designed primarily to decrease contamination originating from wheat germ. The elapsed time for visual evidence of surface contamination did not differ significantly among media (Table 2). There were some significant differences in percentages of rearing containers that became contaminated and in survival from the larval to the adult stages. The lowest incidence of contaminated rearing containers and the highest survival rate of larvae was associated with the AUTOWG+S medium. Although the AUTOWG+S medium was not significantly different from the AUTOWGCS, AUTOWG, or FUMWG media, it was the only medium tested that produced significantly more moths than the CHECK medium. Dry heat sterilization of wheat germ was the least effective method.

The type of medium did not significantly influence the duration of individual stages or the generation time which averaged 47.9±1.1 days (Table 2). The average weights of pupae, however, were significantly different among some of the media. The number of eggs deposited by individual females (average 95.4±12.6, N=176) and percentage hatch (47.5±3.4, N=16,725 eggs) were not significantly (P>0.01) affected by the media.

CONCLUSIONS

Moths of D. amatella were nocturnally active during 12L:12D photoperiods. Female calling behavior and subsequent mating and oviposition were dependent on the presence of host plant material. Mating behavior was delayed for 2 days after emergence and occurred during the last 5 h of scotophase. Multiple matings occurred, but were infrequent.

Survival of D. amatella larvae was optimized when 2 larvae were reared per 30 ml container and microbial contaminants were eliminated. Thus,

### TABLE 2. SURVIVAL FROM FIRST-STAGE LARVAE TO ADULTS, CONTAMINATION OF REARING CONTAINERS, PUPAL WEIGHTS, AND GENERATION TIMES OF Diorhyetria amatella, REARED ON MODIFIED WGCS MEDIUM.

<table>
<thead>
<tr>
<th>Medium**</th>
<th>Average percent survival to adult stage</th>
<th>Avg. No. days to first visible growth</th>
<th>% of rearing containers</th>
<th>Pupal weight (mg)</th>
<th>Generation time (days)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTOWG+S</td>
<td>83.5 a</td>
<td>3.0 a</td>
<td>17.2 a</td>
<td>94.7 a</td>
<td>48.5±4.4</td>
</tr>
<tr>
<td>AUTOWGCS</td>
<td>70.6 a b</td>
<td>21.7 a b</td>
<td>6.1 a</td>
<td>91.5 a</td>
<td>44.0±1.9</td>
</tr>
<tr>
<td>AUTOWG</td>
<td>61.6 a b</td>
<td>28.3 a b c</td>
<td>15.5 a</td>
<td>88.0 a</td>
<td>47.5±1.5</td>
</tr>
<tr>
<td>FUMWG</td>
<td>45.7 a b</td>
<td>43.7 a b c</td>
<td>10.4 a</td>
<td>86.7 a</td>
<td>50.8±3.2</td>
</tr>
<tr>
<td>CHECK</td>
<td>42.5 b</td>
<td>64.5 b c</td>
<td>15.2 a</td>
<td>77.2 b</td>
<td>50.8±3.3</td>
</tr>
<tr>
<td>DRYWG</td>
<td>28.5 b</td>
<td>81.5 c</td>
<td>12.7 a</td>
<td>79.8 b</td>
<td>48.7±9.9</td>
</tr>
</tbody>
</table>

*Any 2 means followed by the same letter are not significantly different at the 1% probability level by Duncan’s multiple range test (N=150-632 larvae, 2-8 replicates).

**AUTOWG:S=wheat germ autoclaved plus ascorbic acid, AUTOWGCS=entire medium autoclaved, AUTOWG=wheat germ autoclaved, FUMWG=wheat germ fumigated, DRYWG=wheat germ dry heat sterilized.

†X±t, 0.01.
semiaspetic techniques should be used when preparing the medium and transferring larvae to rearing containers. Peracetic acid or sodium hypochlorite were equally effective for surface sterilizing eggs; however, sodium hypochlorite is less flammable, easier to store, and more readily available. Sorbic acid should be added to the medium and the wheat germ autoclaved.

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LITERATURE CITED


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EFFECTS OF 'ANTIGUA 2D-118' RESISTANT CORN ON
FALL ARMYWORM¹ FEEDING AND SURVIVAL² ³

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ABSTRACT

The impact of a fall armyworm resistant corn, 'Antigua 2D-118', on larval numbers was measured for 3 years in field studies. The susceptible standard, 'Cacahuacintle Crosses' (Cac.C) had ca. 2 times as many fall armyworms as did 'Antigua 2D-118' 10 days after infestation. The resistance of 'Antigua 2D-118' was expressed in fewer larvae per plant and less leaf damage than the susceptible entry for all 3 years.

RESUMEN

Un estudio de campo de 3 años se evaluaron el efecto sobre el número de larvas de una línea de maíz, 'Antigua 2D-118', la cual es resistente a Heliothis zea (Boddie). Diez días después de la infestación, el estandar susceptible, Cacahuacintle Crosses (Cac.C) tenía cerca de doble el número de larvas que tenía Antigua 2D-112. En cada uno de los 3 años, la resistencia de Antigua 2D-118 se medía en menos larvas por planta y menos daño a las hojas que en el estandar.

Plant resistance to insects has been reported as an ideal control method for insect pests (Luginbill 1969). Dahms (1972) showed the role of plant resistance in an integrated control program by demonstrating the direct and indirect effects of all 3 mechanisms of resistance (nonpreference, antibiosis, and tolerance) and he gave examples of how resistant varieties reduced insect populations. Schalk and Ratcliff (1976) reported an evaluation of programs of the Science and Education Administration (USDA) whereby the use of insect-resistant cultivars has been successful in controlling insect pests and in reducing the use of insecticides. Wiseman et al. (1979) found

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