BOLL WEEVIL REPRODUCTIVE DEVELOPMENT RESPONSES TO CROWDING AND VARIATIONS IN HOST QUALITY

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Abstract

Impacts of crowding and host quality on boll weevil reproductive development and occurrence of diapause characters were investigated under controlled environmental conditions. Crowding and poor host quality influenced fat accumulation, reproductive commitment, and diapause status of females, but did not influence mating activity. Moderation of crowding and improvement in host quality tended to be accompanied by increased reproductive commitment and decreased diapause incidence. Crowding and host quality influenced fat accumulation in males, but did not appear to influence gonadal or seminal vesicle condition. Experimental conditions influenced diapause status of males, but effects were different than for females. Implications of the results to other diapause studies and control programs are discussed.

Introduction

In 1994, the Crop Insects Research Unit of the Subtropical Agricultural Research Laboratory redirected its research efforts and established a 5-yr plan specifically to provide research support to the imminent Boll Weevil Eradication Program in the Lower Rio Grande Valley of Texas. A major component of this research plan is focused on reproductive and survival strategies of subtropical boll weevils because these aspects of boll weevil biology have not been investigated as intensively in the subtropics as in the more temperate cotton production regions.

Previous reports indicate biology and ecology of subtropical populations of boll weevils may not conform to models accepted for other areas. Although Graham et al. (1978) examined typical boll weevil overwintering habitats in the Texas Lower Rio Grande Valley and identified several preferred habitats, relatively few weevils were recovered from these habitats. Overwintering boll weevils in this region are captured in pheromone traps throughout the winter (Guerra and Garcia 1982, Guerra 1983, Wolfenbarger et al. 1976). Mitchell and Hardee (1974) reported that in Mississippi, diapausing weevils tend not to respond to traps. In contrast, Segers et al. (1987) captured weevils in all physiological states, including diapause, throughout most of the year along the Texas lower gulf coast. In a two-year study in the Lower Rio Grande Valley, Wolfenbarger et al. (1976) trapped diapausing weevils in every month except June of 1973 and May of 1974. They also observed that 100% of females and 57-67% of males captured in mid-winter, and ≥50% of females trapped between April and August, were in firm or intermediate diapause. Application of the term diapause to subtropical weevils exhibiting morphological characters described by Brazzel and Newsom (1959) was contested by Guerra et al. (1982) on the basis of the rapid reversion to reproduction that occurred when diapausing weevils were exposed to cotton squares.

Recent investigations at this laboratory, in cooperation with personnel at the University of Texas, have failed to demonstrate the larval induction of diapause in subtropical weevils, or to define titers of juvenile hormone esterase (JHE) that can be used to detect diapause in adults, although relationships between JHE titer and adult longevity were demonstrated (D. W. Spurgeon, unpublished data). This study also indicated that lower temperatures during winter months in the Lower Rio Grande Valley explained much of the difference in longevity among weevil cohorts reared from field-collected fruit between June and November, and identified crowding and food quality as confounding experimental artifacts. Our objective was to examine the effects of crowding and host quality on reproductive development of adult boll weevils under controlled conditions.

Materials and Methods

Adult weevils were reared from infested squares collected between mid-June and mid-July. Squares were held in screen cages in an environmental chamber at 29.4°C and a 13:11 [L:D] photoperiod. Subsamples of squares were periodically opened to assess larval development until ≥75% of larvae had pupated. Pupae were then harvested and placed in petri plates containing a shallow layer of moist vermiculite. Pupae were examined daily until darkened wing pads indicated imminent adult eclosion. Once eclosion began, callow adults were removed from the plates, sexed, and assigned to treatment regimes three times daily.

Because host quality is difficult to quantify, we arbitrarily defined a high-quality host as an uninfested square and a low-quality host an a square in-fested with a boll weevil egg or larva. Crowding was arbitrarily examined at levels of 1, 2, or 4 weevils per square. Thus, weevils were partitioned among four treatment regimes: treatment 1, weevils were placed individually in petri plates with a moistened dental wick and supplied a fresh, uninfested, field-collected square daily; treatment 2, one weevil of each sex was placed in each petri plate and fed as in treatment 1; treatment 3, a pair of weevils of each sex (4 weevils) was placed in each petri plate and fed a freshly picked, infested square (indicated by presence of an oviposition puncture) daily for 3 days, then divided into pairs and fed as in

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treatment 2; treatment 4 was identical to treatment 3 except infested squares were fed daily for 6 days. Weevils in all treatments were maintained at 29.4°C and a 13:11 [L:D] photoperiod.

Ten weevils of each sex were dissected to assess reproductive and fat body condition at 3-d intervals for 6 days after adult eclosion in treatment 1, 9 days in treatments 2 and 3, and 12 days in treatment 4 (only 9 weevils of each sex in treatment 4 were available at 12 days). Weevils were classified as fat if the fat body was hypertrophied and obscured most of the internal organs. Intermediate weevils had well developed fat bodies but substantial portions of the digestive tract or reproductive organs were visible. Weevils were classified as lean if internal organs were generally visible, regardless of the presence or absence of fat. Females were recorded as mated if sperm was detected in either the spermatheca or copulatory pouch. The presence of eggs, mature eggs, and yolk in one or more eggs was noted. Females that oviposited were recorded as having contained a mature egg. Because weevil size was extremely variable, the degree of atrophy of male sexual organs was estimated in relation to body size rather than by direct measurement. Testes were recorded as small if the long axis length was less than 1/2 the combined length of the meta- and mesothorax and the abdomen. Seminal vesicles were classified as small (devoid of sperm or containing sperm but not distended) or large (full of sperm, distended and clearly club-shaped). Females rated as fat and lacking eggs were considered in firm diapause. Females with fat body rated as fat or intermediate and lacking eggs with yolk were considered in intermediate diapause. Males rated as fat and with small yellow testes were considered in firm diapause. Males with intermediate fat body ratings and small yellow testes were considered in intermediate diapause. All statistical analyses were conducted by comparing the frequencies of ratings among treatments in contingency tables using Fisher's Exact Test (EXACT option of the SAS procedure PROC FREQ, SAS Institute, 1988).

Results and Discussion

Fat body ratings of females reflected effects of crowding and food quality (Fig. 1a). Fat body rating did not differ among treatments at 3 d (*P*=0.14) or 6 d (*P*=0.15) after eclosion, but more females in treatment 4 were fat than in other treatments at 9 d after eclosion (*P*<0.01). Early fat accumulation may have been limited because of energy committed to reproductive development in treatments 1 and 2, and by food availability in treatment 2 because during the first several days of feeding the pair of weevils usually consumed all available anthers. Early fat accumulation was probably also limited in treatments 3 and 4 by food availability, because feeding by these weevils typically resulted in consumption of all anthers and considerable portions of the pistil, sepals and developing petals.

The degree of reproductive commitment by females was assessed using the presence of eggs, yolk in females with eggs, and mature eggs. No treatment completely suppressed the production of eggs, but the proportion of weevils with eggs tended to increase with decreasing crowding and increasing food quality (Fig. 1b). More females in treatments 1 and 2 contained eggs after 3 d (*P*<0.01) than in other treatments. Differences among treatments in the proportions of females with eggs could not be demonstrated at 6 (*P*=0.56) or 9 d (*P*=0.67) after eclosion. More females in treatment 1 contained mature eggs after 3 d than in other treatments (*P*<0.01), more females in treatments 1 and 2 contained mature eggs than in treatments 3 and 4 after 6 d (*P*<0.01), and more females contained mature eggs in treatments 2 and 3 than in treatment 4 after 9 d (*P*<0.01, Fig. 1c). Temporal trends in differences among treatments in the proportions of egg-bearing females in which one or more eggs contained visible yolk were similar to the patterns observed for presence of mature eggs (Fig. 1d). These collective data indicate a reproductive response of female weevils to both crowding and food quality. Initial crowding and poor food quality resulted in reduced reproductive commit-ment. Reproductive commitment subsequently increased following modera-tion of these conditions. Comparisons between treatments 1 and 2 also illustrated that the feeding rate of one square per pair of weevils per day was suboptimal for reproductive development. This was also reflected in oviposi-tion behavior. Eight females in treatment 2, 10 in treatment 3, and 6 in treatment 4 oviposited during the experiment. Every ovipositing female was mated and produced at least one fertile egg but only one female produced a normal egg puncture. Many eggs were deposited on the outside of the square or bract or on the petri plate, and punctures from egg deposition within the square were typically unsealed.

Occurrence of the morphological characters of firm or intermediate diapause also was influenced by crowding and food quality (Fig. 1e). Frequency of diapause was higher in treatment 2 than in other treatments at 3 d after eclo-sion (*P*=0.04), higher in treatments 3 and 4 than in treatments 1 and 2 at 6 d (*P*<0.01), and higher in treatment 4 than in other treatments at 9 d (*P*<0.01). Incidence of diapause appeared to be promoted by crowding and poor food quality, and decreased after these conditions were moderated. Low incidence of diapause characters in treatments 3 and 4 at 3 d after eclosion appear to have been caused by the effects of limited food availability on fat accumu-lation.

Unlike other parameters assessed, temporal patterns of mating status were little affected by crowding and food quality (Fig. 1f). The proportion of females mated was low but similar for all treatments at 3 d after eclosion (*P*=1.00). Mating increased considerably by 6 d and continued to increase through 9 d. No differences were observed among treatments at these times (*P*=0.70 and *P*=1.00, respectively). The consistency among treatments in patterns of mating contrast with the marked differences in the patterns of diapause.

Fat body ratings of males also reflected crowding and food effects (Fig. 2a). Fat body ratings did not differ among treatments at 3 d after eclosion ($P=0.73$), but at 6 d fewer weevils in treatments 1 and 4 were fat that in treatments 2 and 3 ($P<0.01$). No differences were observed at 9 d after eclo-sion ($P=0.30$). Energetic costs of replacement of sperm utilized in mating did not cause the relative leanness of weevils in treatment 1 because these weevils were never exposed to females and thus did not mate. Therefore, at least some of the factors influencing fat accumulation appear to differ between the sexes.

In contrast with the results for females, condition of male gonads did not respond to the experimental treatments (Fig. 2b). No differences among treatments in the proportion of weevils with small testes was demonstrated at 3 ($P=0.75$), 6 ($P=0.44$), or 9 d after eclosion ($P=0.49$). Unlike testis size, seminal vesicle condition did vary among treatments (Fig. 2c). No difference among treatments in the proportion of males with large (distended with sperm) seminal vesicles was observed at 3 d ($P=0.31$), but differences were demonstrated at 6 ($P<0.01$) and 9 d after eclosion ($P=0.04$). Temporal patterns of change in seminal vesicle size appeared to be related to mating activity, with increased mating resulting in sperm depletion and a corresponding decrease in seminal vesicle size.

The frequency of occurrence of the morphological characters of diapause was different among treatments only at 6 d after eclosion ($P<0.01$, Fig 2d). Diapause status appeared to be influenced by the treatments, but the relationship was not straightforward. Because of the dependence of the diapause ratings on fat body condition, attainment of the characters of diapause by weevils in treatments 3 and 4 may have been limited by the availability of food, however, this was not the case in treatment 1. Earlier and separate observations indicated that the incidence of diapause characters in weevils held under conditions of treatment 1 increased to about 70% after 9 d. Thus, our data reflect complex and confounding influences of food availability and behavioral responses to crowding. It is also notable that while the frequency of diapause was increasing, considerable mating activity was occurring. Examination of the data for individual pairs of weevils revealed that mating status of females at the time of dissection gave no indication of the expected diapause status of the associated male; all combinations of unmated and mated females and diapausing and non-diapausing males were observed.

Although Keely et al. (1977) previously concluded that food quality was the primary factor controlling boll weevil diapause, it is possible that the characters we used to distinguish diapause did not accurately reflect the physiological states of the weevils. Although we consider the criteria we used for determining diapause status as liberal, our methods were consistent with much of the literature concerning boll weevil diapause (e.g., Lloyd and Merkl 1961, McCoy et al. 1968, Jenkins et al. 1972, Graham et al. 1979, Guerra et al. 1984, and Sivasupramaniam et al. 1995). The relatively short time spans over which we assessed diapause are also well represented in the literature (e.g., Walker 1967, Mangum et al. 1968, McCoy et al. 1968, Carter and Phillips 1974, and Mitlin and Wiygul 1976). If the characters we used were not valid indicators of diapause, our data indicate that other physiological conditions occur that are morphologically indistinguishable from diapause. In this case, the results illustrate the potential for error in similar laboratory studies, and in field studies where the age and history of surveyed weevil populations are not known. If our criteria for assessing diapause were valid, our results reflect differences in the effects of crowding and food quality on diapause responses of the sexes. This possibility was previously proposed by Brazzel and Newsom (1959). More importantly, the responses of females to alleviation of crowding and food availability constraints tends to support previous assertions that reproductive dormancy of subtropical and tropical boll weevils are more accurately described as quiescence rather than a true diapause (Guerra et al. 1982, Guerra et al. 1984). In any event, further investigations of boll weevil reproductive dormancies in the subtropics seems warranted, particularly considering the importance of a comprehensive understanding of this phenomenon to the success of Boll Weevil Eradication Programs in South Texas and northern Mexico.

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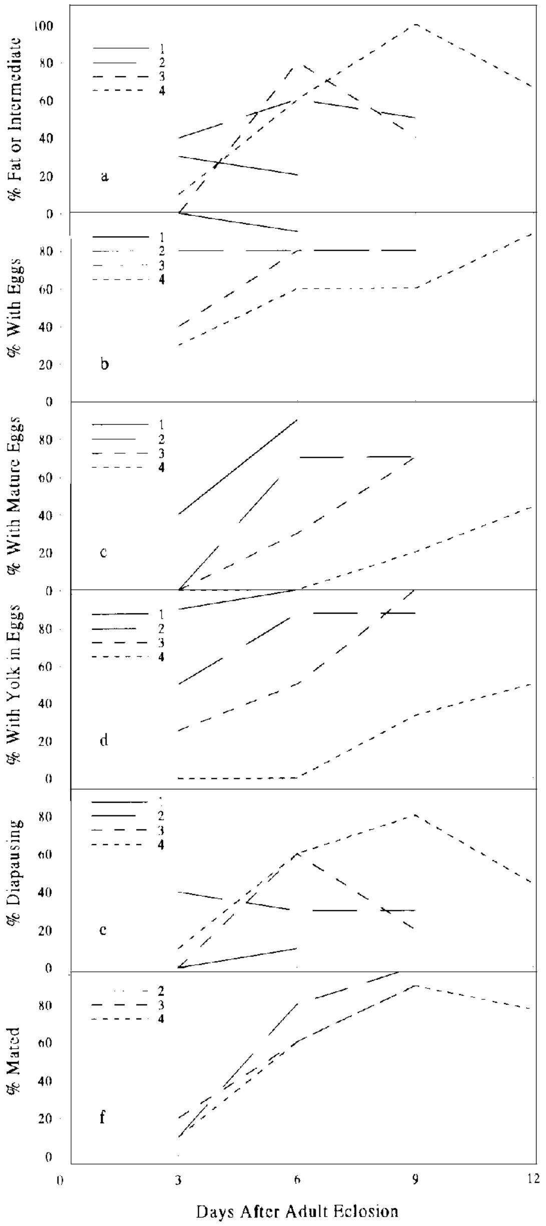

Fig. 1. Effects of crowding and host quality on female boll weevil (a) fat body development, (b) production of eggs, (c) production of mature eggs, (d) occurrence of visible yolk in females with eggs, (e) incidence of diapause, and (f) mating status. Treatments were: (1) individual weevils fed a fresh square daily, (2) one weevil of each sex fed a fresh square daily, (3) two weevils of each sex fed an infested square daily for 3 d, then divided into pairs and fed a fresh square daily, (4) and two weevils of each sex fed an infested square daily for 6 d, then divided into pairs and fed a fresh square daily.

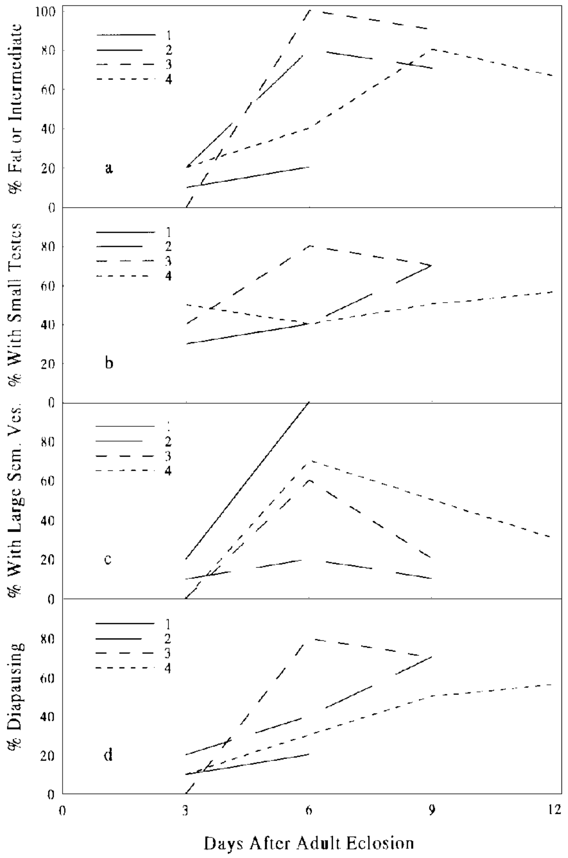

Fig. 2. Effects of crowding and host quality on male boll weevil (a) fat body development, (b) testis condition, (c) seminal vesicle condition, and (d) incidence of diapause. Treatments were: (1) individual weevils fed a fresh square daily, (2) one weevil of each sex fed a fresh square daily, (3) two weevils of each sex fed an infested square daily for 3 d, then divided into pairs and fed a fresh square daily, and (4) two weevils of each sex fed an infested square daily for 6 d, then divided into pairs and fed a fresh square daily.