EFFECT OF FOOD TYPE AND FEEDING RATE ON BOLL WEEVIL DIAPAUSE

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Abstract

Reported results of boll weevil diapause studies have been highly variable, often even within studies. Improved understanding of the sources of this variation would aid efforts to study diapause in the boll weevil. Impacts of selected food characters and feeding regimes on the diapause response were examined in preliminary studies as possible sources of variation. Influence of square source (field versus greenhouse) was examined for weevils fed singly (1 square/weevil daily) and in groups (1 square/5 weevils daily). Field-collected squares tended to be smaller, paler in color, and to have more reddish coloration on the bracts than greenhouse grown squares. Influence of square size was examined by feeding squares with diameters either <7 mm or >7 mm to groups of weevils. Finally, diets of 1 square/5 weevils, 1 boll/10 weevils, or 2 squares plus 1 boll/20 weevils, each replenished 3 times weekly, were examined to identify practical feeding regimes that consistently elicit a high level of diapause. Occurrence of diapause in weevils fed field-collected squares was similar to that in weevils fed greenhouse grown squares. However, differences in square size may have masked the effects of square source because a diet of squares >7 mm resulted in a higher proportion of diapause than did a diet of squares <7 mm. There was no statistical difference in diapause response among diets of squares, bolls, or both. However, diets incorporating bolls tended to provide a numerically greater and more consistent diapause response than did diets of squares alone. These preliminary results suggest the need for stricter control over diets used in boll weevil diapause studies.

Introduction

Recent research regarding diapause induction of the boll weevil in the subtropics has indicated a relative insensitivity of those weevils to photoperiod, but a prominent response to feeding regime (Spurgeon and Raulston 1998). Similar results for weevils from Central Texas have also been obtained (D.W.S., unpublished data). Although other recent reports regarding boll weevils from temperate regions have emphasized the key role of photoperiod in controlling boll weevil reproductive dormancy (Wagner and Villavaso 1999, Wagner et al. 1999), ample evidence of a powerful if not overriding influence of diet exists in the literature (Earle and Newsom 1964, Lloyd and Merkl 1961, Lloyd et al. 1967, Tingle and Lloyd 1969).

One apparent and general characteristic of published results of diapause studies is marked variation in the diapause response among and within studies. The causes of such variation are difficult to identify from the literature because few of these studies adequately describe the feeding regime during the diapause induction period. While most reports distinguish between diets of squares or bolls, comparatively few describe the quantities of fruit fed or frequencies of feeding. Only a single study describes the sizes of squares fed (6 to 10 mm diameter, Spurgeon and Raulston 1998), and experimental evidence to indicate that differences in diapause response would not occur within this range of square size is lacking. Most studies in which food quantity and frequency of food replacement are indicated have used feeding regimes of 1 square per 5 weevils daily or 1 boll per 5 or 10 weevils daily (Harris et al. 1969, Jenkins et al. 1972, Lloyd et al. 1967, Tingle and Lloyd 1969, McCoy et al. 1968). We previously adopted a daily diet of 1 square per 5 weevils as our standard diapause-inducing feeding regime, but despite using a consistent feeding rate and regulating square size, considerable variation in diapause response was observed between replications within experiments (D.W.S., unpublished data).

The variation in diapause response between cohorts of similarly treated weevils is troublesome for several reasons. First, most reports of diapause induction studies represent results of single experiments conducted without replication of cohorts, and without repetition of the experiments to confirm reproducibility of results. Thus, background variation in diapause response increases the degree of difficulty of investigating the diapause phenomenon itself, and may be incorrectly interpreted as effects of photoperiod or temperature treatments. Secondly, we frequently need to rear large numbers of diapausing weevils for release in dispersal, longevity, or overwintering survival studies. Background variation among cohorts reared for these purposes is expensive to quantify because of the large numbers of dissections required, and if differences among cohorts are indicated, we have no means of adjusting or accounting for these effects. Finally, when large numbers of diapausing weevils are required, a daily diet of 1 square per 5 weevils is not practical because undamaged, pesticide-free squares for feeding are scarce in the field for much of the season, and substantial greenhouse resources are required to adequately feed several thousand weevils. Herein we report a series of preliminary experiments intended to examine the contributions of specific food characteristics to variation in the diapause response, and to identify more practical and reliable diapause-inducing feeding regimes worthy of further study.

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Materials and Methods

In all experiments, boll weevil adults of known age were reared from infested squares collected from cotton plants in the field and held in screened cages at 29.4˚C. Squares were inspected for presence of pupae 5 or 6 d after collection. Pupae were removed daily and placed on a thin layer of moistened vermiculite in 100 X 15-mm petri plates. Petri plates were examined at least daily for presence of adults, which were removed and sexed by the tergal notch method (Agee et al. 1964) as described by Sappington and Spurgeon (1999). Weevils were assigned to diet treatments on the day of eclosion. All diet studies were conducted in environmental chambers maintained at 29.4˚C and with a 13:11 [L:D] h photoperiod.

Diapause response was determined by dissection after the feeding period(s) prescribed for each respective experiment, and was dependent on the presence of both hypertrophied fat body and atrophied reproductive organs. Hypertrophied fat body was bright white or yellow, composed of large and well-formed masses, and substantially or completely obscured other internal organs. Ovaries were considered atrophied if no or only previtelligenic oocytes were present. Ovaries containing oocytes with yolk or mature eggs were considered reproductive. Testes were considered atrophied if they were opaque with fat. This fat was typically yellowish to orange in color, but was occasionally whitish, and the testes were usually much smaller than those exhibiting normal reproductive development. Translucent testes with opaque centers indicated spermatogenesis and were considered reproductive, regardless of testis size.

Effects of Square Source: Field Versus Greenhouse

During previous diapause investigations that required a large number of squares for daily maintenance of the experiments, weevils were fed primarily field collected squares which were supplemented by greenhouse-grown squares (D.W.S., unpublished data). However, unpunctured squares collected from the field late in the fruiting cycle tend to be smaller, paler in color, and often have bracteoles with prominent red pigmentation compared with squares produced in the greenhouse. We suspected these characters may have some influence on the diapause response so the influence of square source was examined for weevils fed both singly (1 square per weevil per d), and in groups (1 square per 5 weevils per d).

Weevils fed individually were placed in 100 X 15-mm petri plates on the day of eclosion and supplied daily with a fresh unpunctured square. A short cotton wick (~1 cm) saturated with water was also supplied as a water source. Nineteen females and 22 males each received a fresh field-collected square daily, while 18 females and 21 males were fed squares from the greenhouse. All squares were between 6 and 9 mm diameter, but field collected squares were more frequently in the lower portion of the size range. Also, one or more bracteole of each field collected square was marked by reddish pigmentation. Weevils were dissected after feeding for 9 d.

Weevils fed in groups (12 males and 13 females per group) were held in 473-cm; cardboard cartons with screen lids and fed 5 unpunctured debracted squares (either field collected or greenhouse grown) daily per carton. Water was supplied in a plastic cup with a cotton wick extending from the cup lid. Three cartons (replications) for each square source were used. Weevils assigned to each treatment (square source) eclosed on the same day. At feeding, the total number of squares needed were counted and divided among cartons, taking care to minimize variation in size among groups of squares. Weevils fed in groups were dissected and classed at the same age as were weevils held singly.

Effects of Square Size

Because adequate numbers of unpunctured squares in the upper portion of the 6 to 9 mm diameter size range were not available from the field, we were concerned that differences in square size between the square source treatments might influence the results. Thus, we conducted an experiment to determine the influence of square size on diapause response of weevils fed in groups. Weevils were held in single-sex groups of 40 weevils each and fed daily at a rate of 1 unpunctured debracted square per 5 weevils. Each group was confined in a 473-cm; carton as described for the square source experiment. Twelve cartons of each sex were assigned to each square size treatment (small, 5 to 7 mm diameter; large, >7 to 9 mm diameter). For each combination of sex and square size, 4 cartons (replications) were designated for dissection after each of 7, 14, and 21 d of feeding. At the time of dissection, 20 weevils from each carton were randomly selected to estimate subsequent host-free survival (not reported here) and the remainder were dissected and classed as diapausing or reproductive. Sample sizes for dissections from individual cartons ranged from 9 to 20 and from 11 to 20 for males and females, respectively.

Diapause-Inducing Feeding Regimes

Three feeding regimes were evaluated for use in obtaining large numbers of diapausing weevils. Weevils were held in mixed-sex groups (10 males and 10 females) in previously described cartons. Feeding regimes included: 1) 1 unpunctured debracted square per 5 weevils; 2) 2 squares and 1 boll per 20 weevils; and 3) 1 boll per 10 weevils. Squares and bolls were 6 to 9 mm and 17 to 22 mm in diameter, respectively. Four cartons of weevils (replicates) were assigned to each diet. Weevils were fed 3 times weekly for 9 d and dissected to determine diapause status as previously described.

Statistical Analysis
Differences in diapause status among groups of weevils held singly and fed squares from the field or greenhouse were examined in contingency tables. Data for each sex were arranged in a 2 X 2 contingency table (square source X diapause status) and analyzed using the Fisher exact test (the EXACT option of the SAS procedure PROC FREQ; SAS Institute, 1988).

Differences in diapause status among treatments in which weevils were fed in groups were examined by analysis of variance using the SAS procedure PROC GLM (SAS Institute 1988). In each case, the arcsine-transformed (Zar 1984) proportion of weevils diapausing in each carton was a replicate. The ANOVA models in the experiments examining square source and feeding regime were single factor models. The model in the examination of square size included terms for square size, days fed, and the square size by days fed interaction. Where differences among more than 2 levels of a main effect were indicated, means were separated using the REGWQ option of PROC GLM (SAS Institute 1988).

Results

Effects of Square Source: Field Versus Greenhouse
No difference in diapause response was observed for male weevils held singly and fed either field-collected or greenhouse grown squares for 9 d. No males exhibited the characters of diapause and thus no test was possible or necessary. Square source also failed to result in differences in female diapause (field collected, 21% diapause; greenhouse grown, 22% diapause; P=1.00). Likewise, no difference in diapause response was observed between square sources for weevils of either sex fed in groups (males, field collected=78% diapause, greenhouse=61% diapause, F=1.66; df=1,4; P=0.27; females, field collected=74% diapause, greenhouse=76% diapause, F=0.00; df=1,4; P=0.97).

Effects of Square Size
Square size influenced the diapause response of both sexes of boll weevil (males, F=15.05; df=1,18; P<0.01; females, F=14.38; df=1,18; P<0.01) (Fig. 1), with smaller squares resulting in a lower percentage of weevils in diapause than larger squares. Also, observed proportion of diapause varied with age at dissection (males, F=22.64; df=2, 18; P<0.01; females, F=6.60; df=2,18; P<0.01) (Fig. 1). The square size by days fed interaction was not significant for either sex (males, F=0.63; df=2,18; P=0.55; females, F=3.00; df=2,18; P=0.07). Thus, a differential influence of square size among weevil ages at dissection was not demonstrated.

Diapause-Inducing Feeding Regimes
Feeding regime influenced the percentage of males diapausing after 9 d of feeding (F=5.01; df=2,9; P=0.03) (Table 1) but treatment means could not be separated. A corresponding effect of feeding regime on females was not statistically demonstrated (F=3.55; df=2,9; P=0.07) (Table 1) despite a trend for lower levels of diapause in response to the square diet. Failure to separate means in the male test, and lack of significant differences in the female test were likely caused by poor statistical power because of the considerable variability among replications fed the diet of squares. In general, diets including bolls tended to result in a higher proportion of diapause and less variability among replicates than did the diet of squares.

Discussion

Earle and Newsom (1964) examined the influence of host plant maturity on diapause by feeding weevils squares from plants of different ages, and concluded that host plant maturity did not influence the diapause response. Superficially, our results regarding the effects of square source (field versus greenhouse) on diapause are consistent with their conclusion. However, our findings regarding the influence of square size reveal a potentially important experimental artifact in studies where square size is not carefully controlled in all treatments. Thus, results of our study of square source, and those of other studies using similar methods, may be spurious. Further studies of the impact of square characters related to plant maturity, in which other square qualities such as size are controlled, seem warranted.

The response to square size that we observed was unanticipated in light of reported square size preferences for oviposition. Although we commonly rear adult weevils from field collected squares <7 mm, it is widely accepted that squares of nearly 7 mm (approximately one-third grown) are required for boll weevil reproduction. Cate et al. (1979) indicated that weevils avoided small squares (<7 mm) as oviposition sites. In contrast, McGovern et al. (1987) reported that squares >8 mm were less preferred than smaller squares for oviposition, but these authors did not indicate the lower limit of the square size range that they examined. Wagner et al. (1996) indicated that female weevils show an ovipositional preference for squares measuring 3 to 9 mm, but that squares >7 mm were preferred to smaller squares. Thus, we anticipated that egg production might be promoted by square size classes that are preferred for oviposition. Rather, the proportion of weevils exhibiting reproductive characters was higher for the diet of small squares than for the diet of large squares. These results indicate that in addition to the numbers and frequency of replacement of squares supplied as adult diet in diapause induction studies, the sizes of squares supplied should be controlled as well. Further study under more strictly controlled diets will be required for a more complete understanding of the influence of fruit type and size on boll weevil diapause.

Although the variation among replications in the feeding regime study resulted in analyses with poor power, trends were evident to suggest that higher levels of diapause and reduced variability in the diapause response might be achieved by feeding bolls in preference to squares. Further, the feeding schedule of 3 times weekly is much more practical than schedules requiring daily replacement of the food. We have since used this diet successfully in support of other research objectives, and a more intensive study to further quantify the magnitude and variability of the diapause response to the various diets is planned.

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Table 1. Diapause responses of male and female boll weevils fed specified diets 3 times weekly for 9 d .

Sex	Diet[a]	Mean % Diapause	Standard Error	Range (%)
Male	Square	75.0	11.9	40 – 90
	Boll	97.5	2.5	90 – 100
	Square + Boll	75.0	8.7	60 – 90
Female	Square	50.0	10.8	20 – 70
	Boll	72.5	8.5	50 – 90
	Square + Boll	80.0	4.1	70 - 90

[a]Diets included 1) square, 1 square per 5 weevils, 2) boll, 1 boll per 10 weevils, and 3) square + boll, 2 squares plus 1 boll per 20 weevils

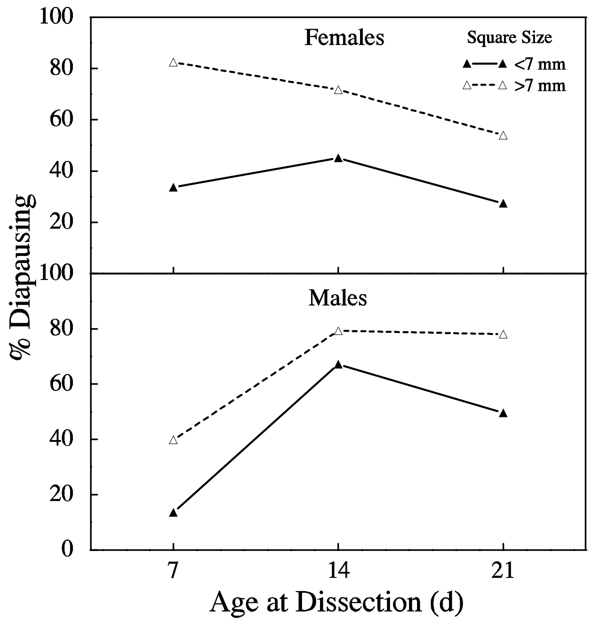

Figure 1. Influence of square size on diapause response of female and male boll weevils fed 1 square/5 weevils daily.