RESPIRATION RATES OF REPRODUCTIVELY ACTIVE AND DIAPAUSING BOLL WEEVILS

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Abstract

Low metabolism is a primary index of diapause in insects. Thus, rates of oxygen (O_2) consumption and carbon dioxide (CO_2) production are lower in diapausing than reproductive insects. To test this hypothesis, a respirometer was used to examine the age-, sex-, and food-dependent respiration rates of reproductive and diapausing boll weevils. CO_2 rates of 97 females and 81 males were measured individually every second over 6-min periods at different ages up to 44 days. Weevils were held at about 27.5°C and fed squares or bolls. Observations indicated that respiration rates of reproductive females declined from about 1.74 to 1.05 μl/mg/hr between day 2 and 44. This decline was attributed to age-related changes in reproductive rates or metabolism. Rates of reproductive males declined in a similar fashion (from 1.50 to 0.76 μl/mg/hr), but reached a lower asymptote (extension of a line approaching a minimum) than females. In contrast, age-dependent rates of diapausing females and males were nearly identical, declining from about 1.73 μl/mg/hr on day 1 to an asymptote of about 0.38 μl/mg/hr. Thus, rates declined more sharply and to a lower asymptote in diapausing than reproductive weevils; e.g., 61% and 54% lower for diapausing than reproductive females and males, respectively. Using 95% confidence intervals to determine the age of separation between reproductive and diapausing adult respiration rates, divergence first appeared on day 3 for females and day 10 for males. The delay in separation for males was due to suppressed respiration rates of reproductive males compared to females. Respiration was more variable in reproductive than diapausing adults probably because of differences in reproductive rates among individuals and a greater capacity for these adults to respond to changing environments. Adult feeding on squares or bolls did not influence respiration.

Introduction

The boll weevil, *Anthonomus grandis grandis* Boheman, uses diapause to aid in overwintering. This physiologically altered state is generally defined by low metabolism, reduced morphogenesis, modified behavior, and heightened resistance to environmental extremes (Tauber et al. 1986). Although diapause is well studied in the boll weevil, many aspects of this complex and dynamic state are poorly understood. For example, little information exists on weevil metabolism, yet this fundamental attribute of diapause can reveal important information on the phases of diapause induction, maintenance, and termination. Indeed, respirometry may even shed light on how the weevil overwinters -- in a state of diapause, *per se*, or in post-diapause quiescence. Previously, Brazzel and Newsom (1959) measured respiration rates of live weevils, reporting O_2 consumption rates 64% lower for diapausing than reproductive adults (0.67 versus 1.85 microliters of O_2 consumed per milligram of body weight per hour, respectively). Lambremont (1961) also reported significantly lower O_2 consumption rates of diapausing versus nondiapausing weevils, measured as homogenized boll weevil tissue. No difference was found between the sexes. These studies did not describe changes in respiration as a function of adult age.

The present study examines age-dependent CO_2 production rates of diapausing and reproductive weevils. It characterizes respiration between the sexes and among adults feeding on squares and bolls. It also serves as preliminary research for assessing respirometry as a means to evaluate diapause induction, maintenance, and termination in the boll weevil.

Materials and Methods

Experimental Procedures

Boll weevils originated from infested flower buds (squares) picked from a cotton field in Washington County, MS on September 5 and 19, 1997. Green infested squares were removed from plants, brought to the laboratory, and divided among clear plastic boxes (27 x 40 x 10 cm) that served as rearing containers. Squares were placed on hardware cloth supported by damp sponges which prevented square drying. Rearing containers were placed in computer controlled cabinets that simulated daily field temperatures and photoperiods of north Mississippi during August, September, and October.

Adult weevils were collected from rearing containers each day of the emergence period. They were sexed on the day of emergence (day 0) and sorted by weight. Weevils <9 mg were discarded. Individual mating pairs were held in 2.5-cm clear plastic cubes containing a paper towel and 1 pristine square (7-10 mm diam) or small boll changed daily throughout the experiment. One to three groups of 8 mating pairs from the same emergence date were tested together in the respirometer (Table 1). Weevils were color coded by sex and number.

A Sable Systems International (Henderson, NV) TR-3 Respirometer was used to measure CO_2 rates of weevils. The system used 16 miniature chambers (Sable Systems Model RC-M) to hold the weevils, connected to two 8-channel multiplexers (Model TR-RM). This setup allowed 15 weevils to be tested sequentially from each group of mating pairs (8 females and 7 males), alternated among chambers by sex starting with female #1 in chamber 2.

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Chamber 1 remained empty, serving to baseline the CO_2 readings of the 15 insects (male #8 was not tested). CO_2 levels were read every second for 3 minutes in the baseline chamber at the beginning and end of each test group, and every second for 6 minutes per weevil, with a 1-minute delay between chambers. Using a diaphragm pump, outside air was pulled through a filter and then pushed through an incubator at 2-4°C to remove moisture. Part of the airstream was then pushed through a column of Drierite® and soda lime (to remove moisture and CO_2) and then through all (inactive) chambers except the one being monitored. The flow rate of this airstream was set at 20-30 milliliters per minute, adding O_2 to and flushing CO_2 from waiting weevils. The remaining airstream was pulled sequentially through a column of Drierite, Ascarite® and Drierite, the active chamber containing the monitored weevil, and the CO_2 analyzer (Model LI-6251, Li-Cor Inc., Lincoln, NB). The flow rate of the sampled airstream was regulated at 50 milliliters per minute using a Sable Systems pump (Model TR-SS1), electronics unit (Model TR-FC1), and mass flow controller valve (Model 840, Sierra Instruments Inc., Monterey, CA). All tests were conducted at about 27.5°C.

Weevils were sampled at intervals from 2 to 9 days, depending on age, for up to 44 days from the day of emergence (Table 1). Of the 178 weevils tested, 64 females and 46 males were classified reproductive or diapausing by dissection (Wagner and Villavaso 1999). The physiological status of 68 weevils were not classifed.

Analytical Procedures

CO_2 rates of weevils were measured in parts per million every second. Data were corrected to baseline levels, converted to microliters of CO_2 produced per milligram of live body weight per hour, and averaged over the 6-minute sampling intervals using Sable Systems DATACAN V® software. Mean CO_2 rates (μl/mg/hr) were then calculated for the 110 dissected weevils sorted by sex, physiological status (e.g., reproduction and diapause), and age. Mean CO_2 rates of reproductive and diapausing females and males were plotted (dependent variable) by age (independent variable), and the logistic equation was fitted to the data for ages>0:

$$F(x) = k/(1+a*\exp(-b*x)) \quad (1)$$

where $F(x)$ = microliters of CO_2 produced per milligram of live body weight per hour for reproductive and diapausing females and males at age x. The constants a, b and k are parameter estimates for the different groups of weevils, determined by nonlinear regression analysis (SAS Institute 1989).

The following method was used to classify the 68 non-dissected weevils as reproductive or diapausing. Models representing reproductive and diapausing females or males were fitted to the observed CO_2 rates for individual non-classified weevils. The Error Sums of Squares (SSE) were calculated (sum of the predicted minus observed values squared) for each physiological status, and the model providing the smallest SSE value determined the physiological status of the individual. If the SSE values of both models were similar, or if they were influenced by outlying observed values, then the observed rates were plotted and visually compared to the predicted rates for each status. The model with the best overall fit was selected to represent the status of the weevil being classified. After all weevils were classified, the mean CO_2 rates (μl/mg/hr) were re-calculated for all weevils sorted by sex, physiological status, and age. Mean CO_2 rates of reproductive and diapausing females and males were then plotted by age, and equation 1 was re-fitted to the data for ages>0. Comparison of respiration rates between sexes and physiological status were made using 95% confidence intervals.

To determine whether adult food influenced respiration, mean CO_2 rates were calculated for groups of weevils sorted by adult food, sex, and status. Equation 1 was fitted to these means, and the results were compared between food groups using 95% confidence intervals.

Results and Discussion

Using equation 1 to establish age-dependent respiration rates of known (dissected) reproductive and diapausing weevils permitted the classification of non-dissected weevils. Combining weevils classified empirically with those classified through dissection had little effect on age-dependent respiration rates (Table 1, compare parameter estimates). Based on overlapping 95% confidence intervals, no differences were detected between like models fit to known weevils and all weevils combined. Thus, models fit to the combined data were used in this study. Similarly, the impact of adult food on respiration rates was undetectable. Based on confidence intervals analysis, no differences were found between square or boll-fed reproductive and diapausing females and males (Fig. 1). Thus, both food groups were pooled for the remaining study.

Respiration rates on the day of emergence (day 0) were suppressed probably because newly emerged adults were unfed at the time of testing, resulting in reduced metabolism (Fig. 2). CO_2 rates peaked on day 1 in diapausing and day 2 in reproductive weevils. These rates were similar between the sexes (about 1.5 to 1.8 μl CO_2/mg/hr). Thereafter, rates declined more sharply and to a lower asymptote in diapausing than reproductive weevils (Fig. 2, dashed versus solid lines). Rates were about 61% and 54% lower for diapausing than reproductive females and males, respectively, measured at the asymptote of the curves (Table 2, parameter *k)*. These percentages are similar to those reported by Brazzel and Newsom (1959), who found a 64% lower O_2 consumption rate for diapausing than reproductive adults.

Age-dependent rates between diapausing females and males were nearly identical (Fig. 3B), declining to an asymptote of about 0.35 to 0.41 μl CO_2/mg/hr (Table 2B and D, parameter *k*). Rates of reproductive weevils differed by sex, with males attaining a 28% lower asymptote than females (Fig. 3A; Table 2A and C, parameter *k*). These differences may be due partly to a higher metabolic cost of egg production. Because males have relatively low rates of CO_2 production, it is more difficult to distinguish their physiological status based on respiration alone. This distinction is protracted to a later age in males compared to females (Fig. 2). For example, a comparison of 95% confidence intervals around the regression lines indicates that CO_2 rates of reproductive and diapausing females diverged starting at day 3. A difference in physiological status in males was first observed on day 10. The declining respiration rates among reproductive weevils may be due to changes in reproductive rates or metabolism associated with aging. Also, Roach (1979) noted reduced fecundity and longevity associated with shortened daylength in females. Weevils in this study experienced decreasing daylengths which may have affected their respiration.

CO_2 rates were more variable in reproductive than diapausing weevils of both sexes (Fig. 2, compare confidence intervals; Table 2, R^2 values). This observation probably indicates varying degrees of reproduction among weevils (e.g., differences in fecundity), and a greater capacity of reproductive adults to respond to changing environments. For example, dramatic spontaneous decreases in respiration rates often were observed when weevils were disturbed during recordings. The higher the CO_2 rate before the disturbance, the greater the decrease, suggesting a greater response of reproductive than diapausing weevils.

These findings revealed important attributes about boll weevil diapause. For example, the similar patterns in respiration rates of diapausing females and males suggests little difference in diapause induction between the sexes. In fact, females and males appear to progress through prediapause at about the same rate. Wagner and Villavaso (1999b) reported similar rates of fat body enlargement in prediapausing females and males. Their model predicted about 4.3, 6.6, and 22.6 days for 1, 50, and 100% of the population to attain hypertrophied fat bodies at 27.5°C. Respiration rates of diapausing weevils declined about 93% during the first 23 days, based on differences in model predictions at day 1 and 44, suggesting a relationship between the heavy feeding period associated with prediapause, fat body enlargement, and the rapid decline in respiration rates. One could hypothesize that weevils should abandon cotton fields and establish overwintering sites after becoming fat but before respiration rates asymptote; but a distinct transition from prediapause to diapause was not detected using respiration rates. Rather, a continuous steady decline in respiration rates was observed, apparently representing an increase in the depth of diapause during the initial 3-6 weeks of adulthood.

References

Brazzel, J.R., and L.D. Newsom. 1959. Diapause in *Anthonomus grandis* Boh. J. Econ. Entomol. 52: 603-611.

Lambremont, E.N. 1961, Homogenate respiration of diapausing and nondiapausing boll weevils. Ann. Enotmol. Soc. Am. 54: 313-316.

Roach, S.H. 1979. Boll weevils: Reproductive potential, feeding, and longevity of overwintering adults, and some effects of photoperiod on fecundity. J. Ga. Entomol. Soc. 14: 346-350.

SAS Institute. 1989. SAS user's guide: statistics. SAS Institute, Cary, N.C.

Tauber, M.J., C.A. Tauber, and S. Masaki. 1986. Seasonal Adaptations of Insects. Oxford Univ. Press, New York.

Wagner, T.L., and E.J. Villavaso. 1999a. Diapause in the Boll Weevil (Coleoptera: Curculionidae): Seasonal Occurrence in Mississippi Populations. Ann. Entomol. Soc. Am. (Accepted 7/28/98).

Wagner, T.L., and E.J. Villavaso. 1999b. Effects of temperature and adult diet on development of hypertrophied fat body in prediapausing boll weevil. Ann. Entomol. Soc. Am. (Accepted 9/8/98).

Table 1. Summary data on collection and emergence dates of test weevils, group assignments, adult food (squares or bolls), number of females and males tested, dissected groups, and the number of times each group was run through the respirometer and at what daily interval.

Collect Date	Emerge Date	Grp	AF	#♀	#♂	Diss [a]	# Samples @ Intervals
Sept 5	Sept 20	A	S	8	7	Y	8 @ 2 d
Sept 5	Sept 20	B	S	8	7	N	8 @ 2 d ; 1 @ 7 d
Sept 5	Sept 20	C	S	8	7	Y	8 @ 2 d ; 3 @ 7 d ; 1 @ 9 d
Sept 5	Sept 21	A	B	8	7	Y	8 @ 2 d
Sept 5	Sept 21	B	B	8	7	N	8 @ 2 d ; 1 @ 7 d
Sept 5	Sept 21	C	B	10	5	Y	8 @ 2 d ; 3 @ 7 d ; 1 @ 9 d
Sept 5	Sept 23	A	S	8	7	N	6 @ 3 d ;1 @ 6 d
Sept 5	Sept 24	A	B	8	7	N	6 @ 3 d ;1 @ 6 d
Sept 19	Oct 5	A	S	8	7	Y	8 @ 2 d ; 4 @ 7 d
Sept 19	Oct 5	B	S	8	7	Y	8 @ 2 d ; 4 @ 7 d
Sept 19	Oct 6	A	B	8	7	Y	8 @ 2 d ; 4 @ 7 d
Sept 19	Oct 6	B	B	7	6	Y	8 @ 2 d ; 4 @ 7 d

a Of the weevils in the dissection groups, 1 female and 7 males died before dissection.

Table 2. Parameter estimates and R^2 values for the logistic equation 1 describing age-dependent CO_2 rates (μl/mg body wt/hr) of dissected and combined (A) reproductive females, (B) diapausing females, (C) reproductvie males, and (D) diapausing males.

Group	*a*	*b*	*k*	R^2
A. Reproductive females				
Dissected	-0.45712	0.12942	1.02626	0.751
Combined	-0.43007	0.12843	1.05295	0.709
B. Diapausing females				
Dissected	-0.80830	0.06443	0.43148	0.953
Combined	-0.82315	0.05774	0.41251	0.971
C. Reproductive males				
Dissected	-0.62092	0.15178	0.73063	0.780
Combined	-0.59325	0.12481	0.76327	0.748
D. Diapausing males				
Dissected	-0.82216	0.04928	0.38347	0.967
Combined	-0.83545	0.04273	0.35414	0.972

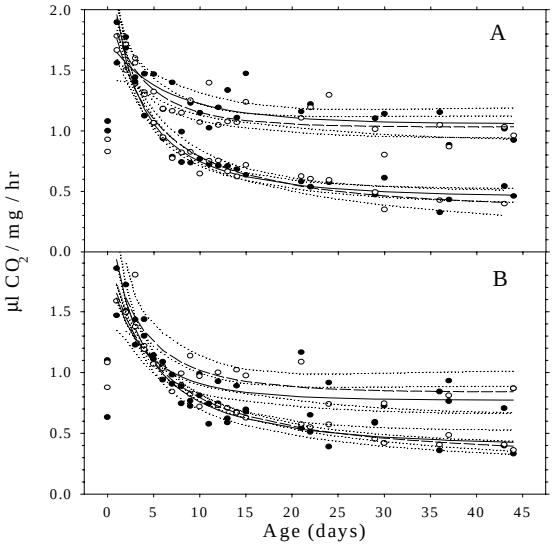

Figure 1. Respiration rates of (A) female and (B) male boll weevils fed bolls (solid dots and lines) or squares (open dots and dashed lines). Reproductive and diapausing weevils are represented by the upper and lower pair of lines and dots, respectively, in plots A and B. Dotted lines represent 95% confidence intervals.

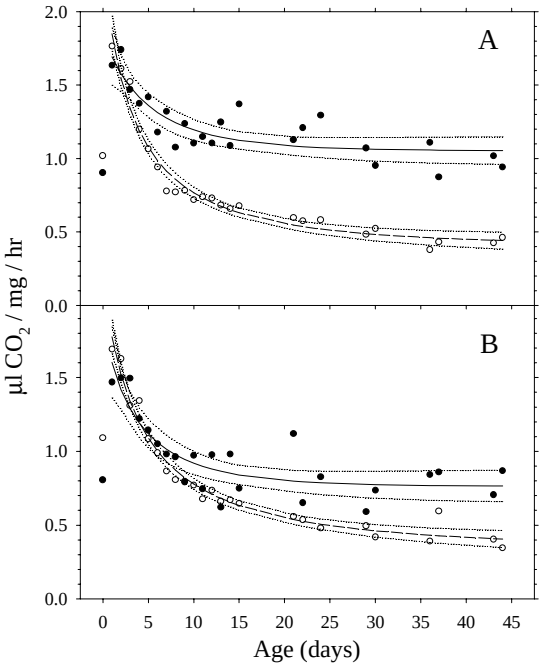

Figure. 2. Respiration rates of (A) female and (B) male boll weevils, reproductive (solid dots and lines) and diapausing (open dots and dashed lines) with 95% confidence intervals (dashed lines).

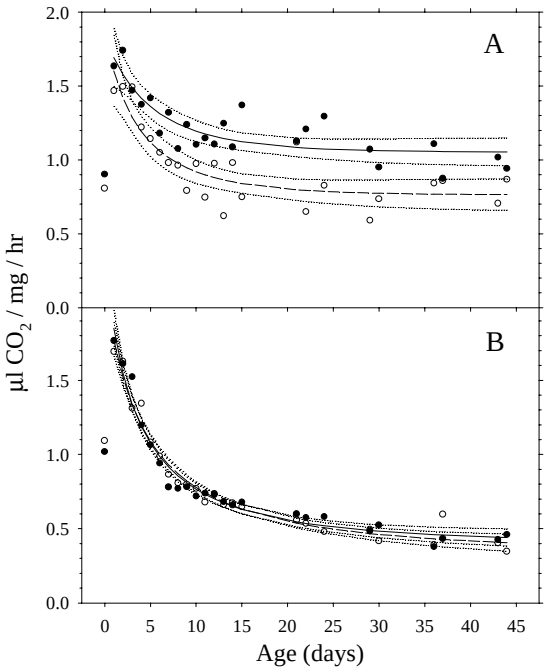

Figure. 3. Respiration rates of (A) reproductive and (B) diapausing boll weevils, females (solid dots and lines) and males (open dots and dashed lines) with 95% confidence intervals (dashed lines).