Mating Behavior of the Bronze Birch Borer, (Coleoptera: Buprestidae)[1,2]

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ABSTRACT Mating behavior of the bronze birch borer, *Agrilus anxius*, was investigated under laboratory and outdoor conditions. Propensity to mate was greater for beetles more than 1-d-old. Duration of copulation tended to increase with increasing beetle age; 87% of mated females were inseminated and subsequently avoided sexual contact. Males mated more than once; previously mated males copulated sooner and for longer periods of time than naive males. Mating frequency did not influence female reproductive success.

KEY WORDS *Agrilus anxius*, bronze birch borer, Buprestidae, mating behavior.

The bronze birch borer, *Agrilus anxius* Gory, is a serious pest of nursery and landscape birch trees, *Betula* species, in North America. Most investigations of this insect have dealt with the damaging larval stage (Balch and Prebble 1940, Anderson 1944, Barter 1957, Ball 1979, Loerch 1983). Adult behavior has rarely been studied.

Mate location by some buprestids is apparently facilitated by host selection instead of pheromones (Carlson and Knight 1969, Matthews and Matthews 1978). Once on the host, mate recognition by *Agrilus* beetles occurs through visual and tactile cues (Carlson and Knight 1969, Gwynne and Rentz 1983, Akers and Nielsen unpublished data). We studied factors that influence mating and reproduction of *A. anxius* to improve our ability to manipulate the beetle as a research organism. This work is necessary to facilitate studies designed to investigate borer/host relationships.

Materials and Methods

Newly emerged, unmated *A. anxius* beetles were collected from European white birch, *Betula pendula* Roth, bolts held in an insectary at The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, (OSU-OARDC) (Akers and Nielsen 1986). Before and after observation periods, beetles were caged communally, according to sex, in crispers containing fresh apple pieces

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and water (Carlson and Knight 1969). Mating behavior was observed at 28 - 32°C on a laboratory bench illuminated by four cool-white fluorescent and two 150-watt incandescent bulbs (1.1×10^3 E $m^{-2}s^{-1}$). Beetles were observed in inverted 30 cm^3 clear plastic cups, 1 male/female pair per cup, during 2 h sessions between 1:00 and 5:00 p.m. (EDST). Preliminary observations indicated that beetles did not mate at night or at low temperature.

Mating frequency vs. age. To investigate the influence of age on propensity to mate, unmated beetle pairs were observed according to age, from less than 1-d-old to 5-d-old. During each observation session, at least five pairs of a given age were observed. Percent of pairs copulating, duration of copulation, and time prior to copulation during each 2-h observation period were recorded. Analysis of variance and Duncan's (1955) new multiple range test ($P = 0.05$) were used to assess the effect of beetle age on these behavioral parameters.

Duration of copulation vs. sperm transfer. The influence of insemination on subsequent mating behavior was investigated. Mating pairs were separated after 1 (four pairs) or 3 (11 pairs) min of copulation or were allowed to uncouple naturally (15 pairs). Sperm transfer was determined by dissecting females and examining spermathecae for sperm.

Influence of previous mating experience. To determine if previous mating experience influences subsequent male/female encounters, beetle pairs were observed for a 2-h period after which they were separated. Beetles that had copulated were then paired again for another 2-h observation period during the next 24 to 48 h. Beetles not copulating were returned to crispers. Mating was observed and recorded as previously described. Percent of beetles copulating in each age category were compared by one-way analysis of variance ($P \leq 0.05$): paired t-tests were conducted to determine if previous mating experience influenced other mating parameters. In other trials to test the effects of previous mating experience, mated males (experienced) were caged with unmated females (naive), and unmated males were caged with mated females. Percent of beetles copulating in the two groups were compared using Student's t-test.

In another experiment, 26 mated pairs were interrupted after 3 min of copulation, separated for 24 h and allowed to mate again. Percent of pairs copulating, length of copulation, and time from pairing to copulation were recorded.

Times prior to copulation and copulation duration between naive, experienced, and 3-min-interrupted beetles were compared to determine the effect of previous mating experience and sperm transfer on mating behavior of beetles 3-d-old and older. Means were separated using Duncan's (1955) new multiple range test.

Influence of mating frequency on reproductive biology. Females that had emerged from *B. pendula* and had already mated one, two, or multiple times (confined with a male continuously) were caged on attached, mature leaves of two adult hosts, either European white birch, or cottonwood, *Populus deltoides* Bartr. ex. Marsh. Two females of each mating frequency (once, twice, or multiple) were caged individually on ten trees/host (six cages/tree) in 19.2 cm^3 transparent plastic petri dishes with top and bottom partially replaced with nylon mesh to provide ventilation. All trees were located at the OSU/OARDC, Wooster. Cages were moved to fresh leaves and examined at least 6 days/week for eggs and dead females. When eggs were found, beetles were moved to a new cage. Cages with eggs were positioned in the interior of a tree canopy and examined three times/ week for egg hatch.

Comparisons of female longevity, fecundity, and egg hatch on each host were analyzed by one-way analysis of variance. Each caged female was considered an experimental unit. Means were separated by Duncan's (1955) new multiple range test ($P = 0.05$).

Results and Discussion

Mating frequency vs. age. Beetle age significantly influenced mating frequency. Beetles 2- to 5-d-old had a 7x higher copulation rate than beetles less than 24-h-old (Table 1). Variability in propensity to mate obscured differences between 1-d-old beetles and those younger or older. However, the most efficient approach for obtaining mated females was to use beetles that were at least 2-d-old.

Duration of copulation/sperm transfer. Duration of copulation was significantly shorter for 2-d-old beetles (348 sec) than for beetles 4- or 5-d-old (432 and 419 sec, respectively); length of copulation tended to increase with increasing beetle age (Table 2). Time from pairing to copulation was not significantly different between age groups.

Duration of copulation influenced sperm transfer. When copulation was interrupted after 1 min, no sperm transfer occurred; at 3 min only 9% of the spermathecae contained sperm. However, when copulation was uninterrupted, 87% of mated females dissected were inseminated.

Table 1. Percent of naive *A. anxius* beetle pairs copulating at different ages under laboratory conditions near Wooster, OH.

Beetle age (days)	No. of 2 h Sessions observed*	$\bar{X}$ % Copulations ± S.D.†
<1	6	4 ± 4 b
1	11	14 ± 12 ab
2	15	27 ± 17 a
3	16	29 ± 18 a
4	10	30 ± 22 a
5	8	29 ± 16 a

* For each session, at least 5 beetle prs. were observed/age group.

† Means followed by the same letter are not significantly different at the $P = 0.05$ level [Duncan's (1955) new multiple range test]. Data were transformed to the arcsine $\sqrt{x}$ before analysis.

Previous mating experience. Previous mating experience had a pronounced effect on the mating behavior of *A. anxius*, but beetle age was not a significant factor in the propensity of 2- and 6-d-old beetles to mate a second time. For beetles of this age range, there was a significant difference in the time from pairing to copulation [(45 ± 23 vs 37 ± 24 min, $\bar{X}$ ± S.D., ($P = 0.1$)] and in duration of copulation [(430 ± 170 vs 488 ± 169 sec ($P = 0.1$)], between first and second copulations, respectively (Student's t-test for paired comparisons).

Mated males caged with unmated females had a higher copulation rate, 45.0 ± 5.8%, than mated females caged with unmated males, 29.6 ± 8.2%, according to Student's t-test ($P = 0.05$). Smith (1970) reported similar results with the peachtree borer, *Synanthedon exitiosa* (Say) (Lepidoptera: Sesiidae). Regardless of age, mated males caged with virgin females copulated sooner than unmated males caged with virgin females. Males mated readily two or more times, but only 58% of the females mated more than once.

Table 2. Duration of copulation and time prior to first copulation of naive *A. anxius* adults under laboratory conditions near Wooster, OH.

Beetle age (days)	No. matings observed	$\bar{X}$ Copulation duration (sec) ± S. D.*	$\bar{X}$ Time prior to copulation (min) ± S.D.†
<1	4	338 ± 110	52 ± 30
1	25	361 ± 104 ab	50 ± 32
2	62	348 ± 89 b	45 ± 34
3	50	376 ± 101 ab	50 ± 36
4	35	433 ± 175 a	57 ± 32
5	36	419 ± 161 a	62 ± 40

* Means followed by the same letter are not significantly different at the $P = 0.05$ level [Duncan's (1955) new multiple range test]. Beetles <1-day-old were not included in the analysis because only 4 matings were observed. Data were transformed to $\sqrt{x}$ before analysis.

† No significant differences at the $P \leq 0.05$ level, ANOVA.

Separating beetles after 3 min of copulation increased their propensity to mate again and reduced the time prior to copulation during the next mating exposure/observation period. Eighty-four percent (± 12%) of the "3-min" beetles mated again during their next opportunity, whereas the highest % copulation for naive beetles was at least 2.8 × lower (Table 1, 4-d-old beetles).

Mating experience and sperm transfer influenced subsequent mating behavior. Time prior to copulation was significantly less for experienced beetles and 3-min-interrupted beetles, 44 and 33 min, respectively, than naive beetles, 56 min (Table 3). Experienced beetles copulated significantly longer, 485 sec, than naive or 3-min beetles, 405 and 411 sec, respectively (Table 3). Although interrupting copulation before sperm transfer resulted in beetles mating sooner at the second meeting, inseminated females were not as receptive and avoided naive males. This difference would seem to be adaptive. Once mated, maturation feeding and oviposition may become dominant activities for females, whereas males contribute most to reproductive success by pursuing additional mates.

Table 3 Comparison of copulation duration and time prior to copulation between naive, experienced, and 3-min-interrupted *A. anxius* adults under laboratory conditions near Wooster, OH.

Beetle mating experience	$\bar{X}$ Time prior to copulation (min) ± S.D.*	$\bar{X}$ Copulation duration (sec) ± S.D.*
Naive	56 ± 36 a	405 ± 145 a
Experienced	44 ± 33 b	485 ± 171 b
3-min-interrupted	33 ± 24 b	411 ± 91 a

* Means in a column followed by the same letter are not significantly different at the $P = 0.05$ level [Duncan's (1955) new multiple range test]. Data were transformed to $\sqrt{x}$ before analysis.

Influence of mating frequency on reproductive biology. Mating frequency did not significantly influence female longevity, fecundity of all females examined, or fecundity of females ovipositing, when beetles were fed *B. pendula* or *P. deltoides* (Table 4). Females mated only once were as fertile as females confined continuously with males. However, beetles caged with *P. deltoides* attached leaves lived longer and laid more eggs than beetles fed leaves of *B. pendula*. Although we did not examine post-ovipositional mating behavior, we did observe beetles copulating in cages attached to leaves.

Table 4. Influence of mating frequency on longevity, fecundity, and egg hatchability of *A. anxius* that emerged from *B. pendula* and fed on birch or cottonwood at Wooster, OH.

Host Plant*	Number of Matings	$\bar{X}$ Longevity (days) ± S.D.	$\bar{X}$ Fecundity ± S.D.	$\bar{X}$ Fecundity ± S.D. of ovipositing females	$\bar{X}$ % Egg Hatch ± S.D.
	1	16.4 ± 4.4	1.5 ± 3.6	5.6 ± 5.5	11.3 ± 21.8
Betula pendula	2	16.8 ± 5.4	1.0 ± 1.6	3.0 ± 1.3	15.8 ± 20.1
	Multiple†	18.0 ± 5.4	1.2 ± 3.5	7.3 ± 6.5	23.8 ± 21.8
	1	30.8 ± 15.5	10.3 ± 17.0	19.2 ± 19.4	23.5 ± 21.9
Populus deltoides	2	31.5 ± 13.0	5.2 ± 10.2	9.1 ± 12.4	19.6 ± 38.0
	Multiple	32.1 ± 11.2	9.0 ± 13.5	14.2 ± 14.7	7.0 ± 11.7

* Means within a column for a host plant are not significantly different at the $P \leq 0.05$ level, ANOVA. Data for $\bar{x}$ longevity, fecundity, and fecundity of ovipositing females were transformed to $\sqrt{x}$ before analysis; data for $\bar{x}$ % egg hatch were transformed to arcsine$\sqrt{x}$ before analysis.

† Females caged continuously with male.

Summary

Beetle age and previous mating experience had the greatest impact on *A. anxius* mating behavior. Using unmated females at least 3-d-old and "experienced" males decreased the time required to obtain mated females for experimentation.

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References Cited.

Akers, R. A. and D. G. Nielsen. 1986. Influence of post-felling treatment of birch logs on emergence success of bronze birch borer, *Agrilus anxius*, adults (Coleoptera: Buprestidae). J. Entomol. Sci. 21: 63-67.

Anderson, R. F. 1944. The relation between host condition and attacks by the bronzed birch borer. J. Econ. Entomol. 38: 588-596.

Balch, R. E. and J. S. Prebble. 1940. The bronze birch borer and its relation to the dying of birch in New Brunswick Forests. For. Chron. 16: 179-201.

Ball, J. 1979. Interactions of bronze birch borer (*Agrilus anxius* Gory) and European white birch (*Betula pendula* Roth) populations in urban environments. M.S. thesis, Mich. State Univ., E. Lansing.

Barter, G. W. 1957. Studies of the bronze birch borer, *Agrilus anxius* Gory, in New Brunswick. Can. Entomol. 89: 12-36.

Carlson, R. W. and F. B. Knight. 1969. Biology, taxonomy, and evolution of four sympatric *Agrilus* beetles (Coleoptera: Buprestidae). Contrib. Am. Entomol. Inst. 4: 1-105.

Duncan, D. B. 1955. Multiple range and multiple *F.* tests. Biometircs 11: 1-42.

Gwynne, D. T. and D. C. F. Rentz. 1983. Beetles on the bottle: male buprestids mistake stubbies for females (Coleoptera). J. Aust. Entomol. Soc. 23: 79-80.

Loerch, C. R. 1983. Instar determination, within tree distribution and natural enemies of immatures of the bronze birch borer, *Agrilus anxius* Gory (Coleoptera: Buprestidae), in Pennsylvania. M. S. thesis. Penn. State Univ., University Park.

Matthews, R. W. and J. R. Matthews. 1978. Insect behavior. Wiley, New York.

Smith, C. F. 1970. Sexual activity and egg fertility of the peach tree borer. J. Econ. Entomol. 63: 1909-1910.