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MATING BIOLOGY OF *AUSTROMUSOTIMA CAMPTOZONALE* (LEPIDOPTERA: CRAMBIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF OLD WORLD CLIMBING FERN, *LYGODIUM MICROPHYLLUM* (SCHIZAEACEAE)

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ABSTRACT

Austromusotima camptozonale (Hampson) is under investigation as a potential biological control agent of Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br., which is a serious invasive weed in southern Florida. Studies were conducted to investigate aspects of the mating biology of *A. camptozonale* with a view to improving field colonization efforts. A laboratory colony of *A. camptozonale* had a female-biased sex ratio of 1:1.3 male:female, with females emerging slightly earlier than males. The majority of female moths mated only once, even when they were confined with multiple males for several nights. Sex ratio had a significant effect on the percentage of females that were mated, with higher percentages of females mated at high male sex ratios. However sex ratio had no effect on the number of times individual females mated. Larval production was significantly higher in colony cages with high male sex ratios, and this was likely due to the higher percentages of females that were mated in these cages. Data suggest that *A. camptozonale* females are likely to be functionally monandrous under field conditions. Females produced a lifetime average of 61.2 ± 9.7 larvae, and were short-lived, surviving an average of 5.7 ± 0.5 d. Females began oviposition on the first night after mating and continued until the day of their death. However 90% of eggs were deposited between the first and third night after mating. Due to the short lifespan of female moths, adults may not be the best life stage for field release in a biological control program, owing to likely disruption of critical mating and oviposition activities. Elevating the ratio of males in colony mating cages is a strategy for maximizing female reproductive output.

Key Words: *Austromusotima camptozonale*, mating biology, *Lygodium microphyllum*

RESUMEN

Austromusotima camptozonale (Hampson) esta bajo investigación como una agente potencial de control biológico para el helecho trepador de Mundo Antiguo, *Lygodium microphyllum* (Cav.) R. Br., que es una maleza invasora seria en el sur de la Florida. Se realizaron estudios para investigar la biologia del apareamiento de *A. camptozonale* con el propósito de mejorar los esfuerzos de colonización de esta especie en el campo. Una colonia de laboratorio de *A. camptozonale* tuvo una preferencia en la proporción de machos a hembras de 1:1.3 macho:hembra, con las hembras emergiendo un poco antes que los machos. La mayoria de las palomillas adultas se aparearon solamente una vez, aun cuando fueron confinadas con machos multiples para varias noches. La proporción de macho a hembra tuvo un efecto significativo sobre el porcentaje de hembras que se aparearon, con un mayor porcentaje de hembras apareadas cuando las proporciones de macho a hembra fueron mas altos. Sin embargo, la proporción de macho a hembra no tuvo ningún efecto sobre el numero de veces que las hembras individuales se aparearon. La producción de larvas fue significativamente mas alta en las colonias en jaulas que tuvieron una proporción mas alta de machos a hembra, y esto probablemente es debido a la proporción mas alta de hembras que fueron apareadas en estas jaulas. Estos datos indican que las hembras de *A. camptozonale* son probablemente funcionalmente monógamas bajo condiciones de campo. Las hembras producen un promedio de 61.2 ± 9.7 larvas por todo su vida, y vivieron por un tiempo corto, sobreviviendo por un promedio de 5.7 ± 0.5 dias. Las hembras empezaron la oviposición en la primera noche después de aparearse y continuaron hasta el dia de su muerte. Sin embargo, 90% de los huevos fueron depositados entre el primer y tercer dia despues del apareamiento. Debido a la duración corta de vida de las palomilla hembras, los adultos posiblemente no son el mejor estadio para hacer liberaciones de esta especie en el campo en un programa de control biologico, probablemente a causa de la disrupción crítica en las actividades del apareamiento y oviposición. El aumento de la proporción de machos en las colonia usadas para el apareamiento en jaulas es una estrategia para maximizar el rendimiento reproductivo de las hembras.

A variety of different mating systems are found within lepidopteran species (Drummond 1984; Arnqvist & Nilsson 2000). These systems range from monandry, in which females mate with only a single male during their lifetime, as occurs in the gypsy moth (Giebultowicz et al. 1991), to systems where females exhibit a high degree of polyandry and mate with many different males, as occurs in the corn earworm (Raina et al. 1986) and some arctiids (LaMunyon 1997). Although a single mating is often sufficient to provide a female with a lifetime of sperm, polyandry is common in Lepidoptera (Arnqvist & Nilsson 2000; Ridley 1990). Polyandry confers a variety of benefits to females including full fertilization of her egg complement, increased genetic diversity of offspring, receipt of non-sperm nutritional benefits, and avoidance of problems with old sperm (Drummond 1984; Ramaswamy et al. 1997; Jimenez-Perez et al. 2003). However there are also costs associated with polyandry including; energetic costs of courtship, energetic costs of multiple matings, reduced time for oviposition, increased predation risk, and increased risk of pathogen transfer (Svensson et al. 1998; Jimenez-Perez et al. 2003).

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br., is native to tropical regions of Africa, Australia and Southeast Asia (Pemberton 1998). Sometime during the latter half of the 20th century, *L. microphyllum* became established in southern Florida (Pemberton & Ferriter 1998), and over recent decades has spread rapidly to infest communities on moist soils on private lands, parks, wildlife refuges, and even remote locations in Everglades National Park (Volin et al. 2004). *Lygodium microphyllum* grows rapidly under the subtropical conditions of southern Florida, climbing over native plants, denying them light, and smothering them. *Lygodium microphyllum* readily grows up trees, providing a conduit by which brush fires may move into tree canopies, where damage may be done even to mature trees (Pemberton & Ferriter 1998; Volin et al. 2004). Most natural areas in south central Florida are vulnerable to invasion by *L. microphyllum* (Pemberton & Ferriter 1998) and consequently this weed poses a substantial threat to native plants and ecosystems (Lott et al. 2003).

Lygodium microphyllum has proven to be extremely difficult to manage in Florida (Hutchinson et al. 2006). Herbicide treatments are not effective at preventing re-growth and mechanical removal is too expensive to be viable. In addition, native insect and arthropod herbivores are rarely encountered feeding on *L. microphyllum*, so the fern is not subject to the feeding pressures that typically help to regulate plant populations within their home range. Biological control is being considered as a potential management solution (Pemberton 1998). The United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, Florida, in cooperation with overseas collaborators has conducted surveys in SE Asia and Australia to identify host-specific arthropod herbivores of *L. microphyllum* that could be introduced to southern Florida to help control Old World climbing fern (Goolsby et al. 2003). One potential biological control agent is a moth, *Austromusotima camptozonale* (Hampson) (formerly *Cataclysta camptozonale*) (Lepidoptera: Crambidae) (Yen et al. 2004). Larvae of *A. camptozonale* are defoliators of *L. microphyllum*, and are thought to reduce the fern's capacity for vegetative growth and reproduction.

In Dec 2004, the USDA Animal Plant Health Inspection Service (APHIS) issued a release permit for *A. camptozonale*, and field releases of adult moths were made by USDA-ARS-IPRL during the spring and summer of 2005. However subsequent monitoring of release sites during fall 2005 failed to yield definitive evidence of moth establishment. In an effort to better understand the biology of *A. camptozonale* and improve the success of future field colonization efforts, studies were conducted to elucidate aspects of the mating biology and life history traits of adult *A. camptozonale*. Studies were conducted to investigate patterns of adult emergence, sex ratio, male and female longevity, the timing of oviposition, and effect of different sex ratios on mating frequency and female fertility.

Materials and Methods

Insects

Insects used in studies were obtained from a laboratory colony of *A. camptozonale* maintained at the USDA-ARS Invasive Plant Research Lab in Fort Lauderdale, FL. This Fort Lauderdale colony was derived from a pre-existing USDA colony maintained at the Florida Department of Agriculture & Consumer Services, Quarantine facility in Gainesville, FL. The Gainesville colony had been established from insects collected in Apr 2000 from Carbrook Creek, Brisbane, Queensland, Australia.

Colony Maintenance

Larvae in the Fort Lauderdale colony were fed fresh *L. microphyllum* foliage collected weekly from infested field sites in Palm Beach and Martin counties in southern Florida. Immediately following collection, plant material was soaked for 10 min in 10% (v/v) Clorox® bleach in water, and subsequently rinsed 5 times in clean water. Washed foliage was drained and packaged in clean 18-liter plastic bags, and stored at 4°C until needed. Larvae were reared in 739-mL, plastic sandwich boxes (Glad Products Company, Oakland, CA)

(25 larvae per box) on fresh-cut *L. microphyllum* foliage, at 24°C and a photoperiod of 12:12 (L:D). Larvae were allowed to pupate in rearing containers, and following emergence, adults were collected in a bench top light box, with a battery-powered, handheld aspirator (Hausherr's Machine Works, Toms River, NJ). Male moths (prominent dark brown stripes on wings) and female moths (fewer, paler stripes, prominent wing spots) were sexed visually (Yen et al. 2004) and transferred into aluminum-framed mating cages (61 × 30 × 30-cm) screened with insect-proof, metal window screen. Mating cages were positioned on the bench top, and were provisioned with an oviposition sprig, consisting of a bouquet of *L. microphyllum* stems wrapped in cotton balls and inserted into a 150-mL plastic vial (Thornton Plastics, Salt Lake City, UT) of water, and 2 cotton balls, 1 soaked in 9:1 Gatorade® [Lemon-Lime]:honey, the other in water. Gatorade® contains sucrose, glucose, and fructose. These sugars and honey have frequently been used to supplement diets of adult female Lepidoptera in captivity as a source of sugars, amino acids, and vitamins they might otherwise have obtained from extra floral nectaries or homopteran honeydew in the environment (Romeis & Wackers 2002; Tisdale & Sappington 2001). Moths were allowed to mate and oviposit, and following their death, oviposition sprigs were removed and incubated in clear plastic boxes (11 × 11 × 23-cm) (Pioneer Plastics, Inc., Dixon, KY) on the bench top until larvae emerged. Newly emerged neonate larvae were transferred with a paintbrush onto fresh *L. microphyllum* foliage in sandwich boxes to begin the next rearing cycle. *Austromusotima camptozonale* is multivoltine and so can be reared continuously in the lab. Typical durations for the egg, larval, and pupal stages at 24°C are 10, 10, and 11 d, respectively (A. J. B., unpublished data).

Emergence and Sex Ratio Studies

Sandwich boxes containing *A. camptozonale* larvae were selected at random from the colony and put aside for use in adult emergence and sex ratio studies. Following pupation, pupae were transferred into individual 25-mL plastic vials (Thornton Plastics, Salt Lake City, UT), containing a small (1 × 1-cm) piece of moistened paper towel. Vials were left on the bench top and checked daily for emergence, and the numbers of male and female moths recorded. Observations were repeated over 5 larval cohorts, and a total of 347 pupae.

Colony Cage Studies

Experiments were conducted to investigate the effect of the ratio of male to female moths inside mating cages (henceforth "cage sex ratio") on subsequent larval production by female moths. Aluminum mating cages provisioned with oviposition sprigs and cotton balls as previously described, were set up with 1 of 3 ratios of virgin male to virgin female moths: (i) 5 male (♂): 10 female (♀); (ii) 10♂:10♀; (iii) 20♂:10♀. Cages were set up at the same time and were left undisturbed on the bench top for several days to allow time for female moths to mate and oviposit. When all female moths had died, oviposition sprigs were removed from cages and transferred to individual clear plastic boxes on the bench top until eggs emerged. The number of neonate larvae on each sprig was determined and used as a measure of total female oviposition at that cage sex ratio. Studies were set up according to a replicated complete block design, with each of the 3 cage sex ratios replicated across 5 generations of the colony. In 2 of these replicates, female moths were dissected after death. The numbers of spermatophores present in the bursa copulatrix of each female was used to infer how many times she had mated (Drummond 1984).

Individual Female Studies

Experiments with a single female moth per mating cage were performed to more precisely investigate the effect of male to female sex ratio on the frequency of female matings and subsequent fertility. Large, clear-plastic mating cages (41 × 41 × 41-cm) with access sleeves on either side, provisioned with an oviposition sprig and food-soaked cotton balls as previously described, were set up on the bench top with newly emerged virgin female and virgin male moths to achieve 1 of 3 cage sex ratios: (i) 1♂:1♀; (ii) 2♂:1♀; (ii) 3♂:1♀. Cages were left overnight for females to mate, and on the morning of the second day, male moths were removed from cages and oviposition sprigs were replaced. Oviposition sprigs were replaced daily until females died. Oviposition sprigs were maintained in plastic boxes on the bench top until eggs hatched, at which point the number of larvae on each sprig was determined. Following death, female moths were dissected and the number of spermatophores present within the bursa copulatrix was noted. Experiments were set up according to a complete block design, with treatments replicated 10 times.

Individual Male Studies

Experiments were set up to assess the propensity of male moths to mate with more than 1 female moth. A single, newly emerged virgin male moth together with 2-5 newly emerged virgin female moths were added to each of 5 plastic insect rearing cages (30 × 30 × 30-cm) (BioQuip Products, Rancho Dominguez, CA). Each cage was provisioned with an oviposition sprig and cotton balls

soaked in liquid food, as previously described. Moths were left in cages overnight to mate and the next day females were removed and replaced with new virgin females. Females were replaced daily until male moths died. Upon removal, female moths were dissected and the number of spermatophores in each female was recorded.

Statistical Methods

Data were analyzed according to the General Linear Models for analysis of variance (ANOVA). Data were checked for conformity to ANOVA's underlying assumptions of normality of error and homogeneity of variance by examining plots of residuals and predicted values, and when necessary data were transformed to fix departures from these assumptions. Data on percentage of females mated, spermatophore counts, mean larval production and adult longevity data were analyzed by using the univariate ANOVA procedure of SPSS statistical software (SPSS, Inc., Chicago, IL) with post-hoc comparison of means with Tukey's means separation test. Data on daily oviposition across sex ratios were analyzed by the repeated measures ANOVA procedure of SPSS. Emergence data for male and female moths were combined across replicates, and used to construct Kaplan Meier survival curves for cumulative daily emergence. Dead pupae were excluded from consideration. Survival curves were then compared by a log-rank test to check for statistical differences in the timing of emergence between male and female moths.

Results

Emergence and Sex Ratio Studies

Adults generally started emerging 10 to 11 d after pupation, but considerable variability was present in the timing of emergence of moths across cohorts. For this reason, daily emergence data for the different cohorts was synchronized relative to the first day on which adults started to emerge (Fig. 1). Moths emerged over a period of 7 d, with peak emergence occurring on the second, third and fourth days. Both sexes were present from the first day of emergence, but females showed a small but statistically significant tendency to emerge earlier than males (log rank statistic = 11.67; $df = 1$; $P = 0.0006$). Sex ratios of males to females across the 5 cohorts ranged from 1:0.9 to 1:1.5, with an overall ratio of 1:1.3 male:female, respectively (Table 1). Overall adult emergence from the pupal stage was 88.4%.

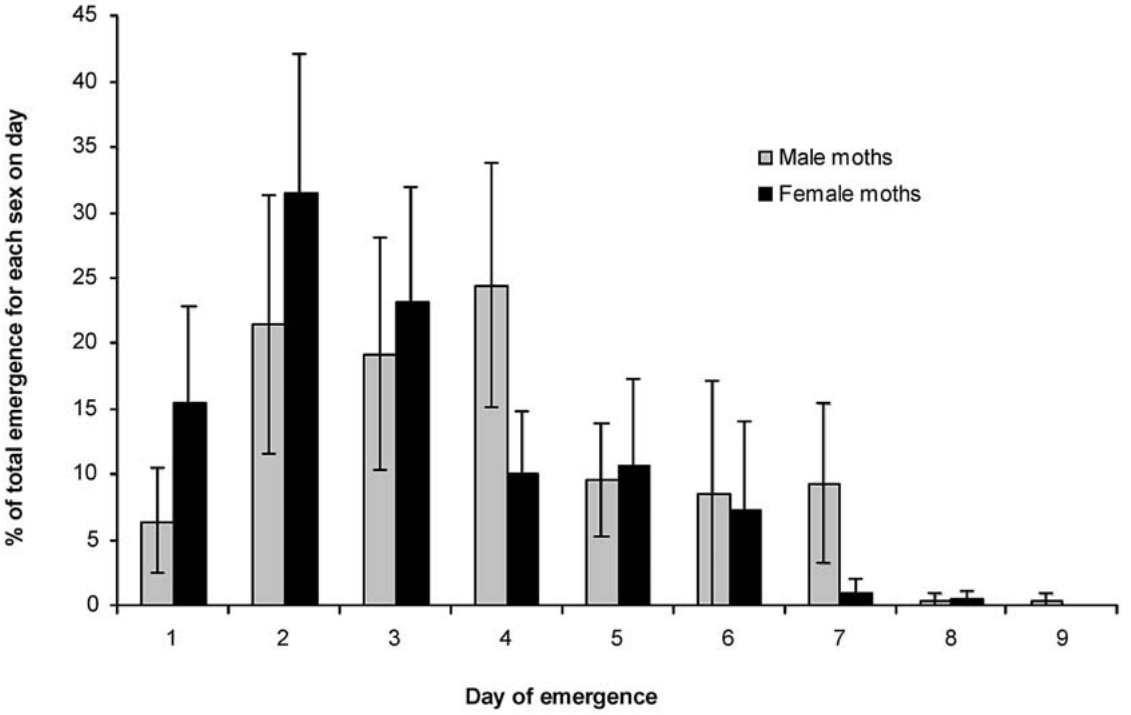

Fig. 1. Timing of emergence of male *A. camptozonale* moths relative to female moths. Graph shows percentage of total emergence for each sex occurring on the first through ninth d of the emergence. Figure constructed by averaging percent daily emergence data for males and females from 5 pupal cohorts ($n = 155$ ♂; $n = 192$ ♀). Emergences across cohorts synchronized relative to the first d on which adults emerged. Bars show standard errors.

TABLE 1. SEX RATIO AND PERCENTAGE EMERGENCE OF MOTHS.

Cohort	Number ♂ moths	Number ♀ moths	Number moths that died	Sex ratio ♂:♀	% Adult emergence[1]
1	10	14	1	1:1.4	96.0
2	10	15	0	1:1.5	100.0
3	9	12	4	1:1.3	84.0
4	91	118	31	1:1.3	87.1
5	35	33	23	1:0.9	74.7
			Overall	1:1.3	88.4

[1]Percentage of pupae from which adult moths emerged.

Colony Cage Studies

Cage sex ratio had a significant effect on mean percentage of females that were mated ($F = 35.95$; $df = 2, 6$; $P = 0.03$) (Table 2). Tukey's test indicated that a significantly higher percentage of females were mated in cages at the 20♂:10♀ ratio, than were mated in cages with ratios of 5♂:10♀ or 10♂:10♀. However, cage sex ratio failed to explain a significant amount of the variation seen in mean number of spermatophores per mated female ($F = 1.26$; $df = 2, 36$; $P = 0.30$) (Table 2). Mean number of spermatophores per mated female in cages at the 20♂:10♀ ratio was not significantly higher than mean number of spermatophores per female observed in either the 5♂:10♀ or the 10♂:10♀ cages. Colony cage larval production data were transformed by squaring to stabilize the variance, and analyzed according to a blocked ANOVA design. Sex ratio was shown to have a significant effect on mean larval production ($F = 5.77$; df 2, 15; $P = 0.03$). The back-transformed mean and standard error for cages receiving the 5♂:10♀ ratio (367.2 larvae/cage ± 222.0) was not statistically different from cages receiving the 10♂:10♀ (337.8 larvae/cage ± 149.0) ratio. Mean larval production for cages with a sex ratio of 20♂:10♀ (498.1 larvae/cage ± 250.0) was substantially higher than mean larval productions observed in cages with 10♂:10♀ or 5♂:10♀, although this difference was only statistically significant between the 20♂:10♀ and 10♂:10♀ treatments.

Individual Female Studies

Although there was a trend towards increasing percentages of mated females at high male-biased sex ratios, the effect was not statistically significant ($F = 2.10$; $df = 2, 27$; $P = 0.14$) at the sample sizes used in these studies (Table 3). Sex ratio did not have a significant effect on mean number of spermatophores per mated female ($F = 0.79$; $df = 2, 23$; $P = 0.47$) (Table 3). Based on 10 replicates of the single-female studies, mean larval production values at the 1♂:1♀, 2♂:1♀, and 3♂:1♀ sex ratios were 73.0 ± 17.8, 56.0 ± 20.0, and 54.6 ± 13.6 larvae/female, respectively, and mean female longevity at these sex ratios were 5.8 ± 0.7, 5.5 ± 0.9, 5.9 ± 0.9 d, respectively. Cage sex ratio did not have a significant effect on mean larval production per female ($F = 0.287$; $df = 2, 24$; $P = 0.76$) or mean female longevity ($F = 0.048$; $df = 2, 21$; $P = 0.95$) and means were not significantly different across treatments by Tukey's means separation

TABLE 2. DISSECTION DATA SHOWING EFFECT OF SEX RATIO ON FREQUENCY OF MATING AND SPERMATOPHORE TRANSFER IN FEMALES MAINTAINED IN COLONY CAGES.

		Percentage of females (Mean ± S.E.)				
Cage ratio (♂:♀)	No. females[1]	Unmated	Once mated	Twice mated	Mated once or more[2]	Spermatophores/ mated female (Mean ± S.E.)[3]
5:10	16	31.3 ± 6.3	68.7 ± 6.30	0.0 ± 0.0	68.7 ± 6.3 a	1.00 ± 0.00 a
10:10	19	36.7 ± 3.3	57.8 ± 2.20	5.6 ± 5.6	63.4 ± 3.3 a	1.08 ± 0.08 a
20:10	17	5.0 ± 5.0	80.0 ± 20.0	15.0 ± 15.0	95.0 ± 5.0 b	1.19 ± 0.10 a

[1]Females from 2 replicates of the colony cage studies (10 ♀ per treatment, per replicate) were dissected. In a small number of cases, dissections were ambiguous revealing the presence of material in the bursa copulatrix that could not be definitively identified as a spermatophore. These females were excluded from consideration.

[2]Means followed by different letters are significantly different by Tukey's means separation test ($P < 0.05$).

[3]Mean calculated as "total number of spermatophores" divided by "number of mated females".

TABLE 3. DISSECTION DATA SHOWING EFFECT OF SEX RATIO ON FREQUENCY OF MATING AND SPERMATOPHORE TRANSFER IN STUDIES WITH SINGLE FEMALES.

Cage ratio (♂:♀)	No. females[1]	Percentage of females (Mean ± S.E.)				
		Unmated	Once mated	Twice mated	Mated once or more[2]	Spermatophores/ mated female (Mean ± S.E.)[3]
1:1	10	30.0 ± 15.3	70.0 ± 15.3	0.0 ± 0.0	70 ± 15.3 a	1.00 ± 0.00 a
2:1	10	10.0 ± 10.0	90.0 ± 10.0	0.0 ± 0.0	90 ± 10.0 a	1.00 ± 0.00 a
3:1	10	0.0 ± 0.0	90.0 ± 10.0	10.0 ± 10.0	100 ± 0.00 a	1.10 ± 0.10 a

[1]Females from 10 replicates of the single female studies (1 ♀ per treatment, per replicate).
[2]Means followed by different letters are significantly different by Tukey's means separation test ($P < 0.05$).
[3]Mean calculated as "total number of spermatophores" divided by "number of mated females".

test ($P > 0.05$). When data were pooled across sex ratios, mean number of spermatophores per mated female was 1.04 ± 0.04, mean larval production was 61.2 ± 9.7, and mean female longevity was 5.7 ± 0.5 d.

Repeated measures ANOVA identified "days after mating" as a factor explaining significant amounts of variation in nightly oviposition totals ($F = 27.28$; $df = 1, 21$; $P < 0.01$). However, repeated measures ANOVA failed to identify sex ratio as a factor explaining significant variation in nightly oviposition totals ($F = 0.35$; $df = 2, 21$; $P = 0.71$), so oviposition data from females at the 3 different sex ratios were combined. Data showed that no eggs were laid during the first day/night that female and male moths were together (Fig. 2). Oviposition began and was highest on the second day/night after the female and male moth(s) were put together, averaging 20.8 eggs, decreasing through 18.5, 16.1, 3.8, 1.7, and 0.3 eggs per day/night during the third, fourth, fifth, sixth, and seventh days/nights, respectively.

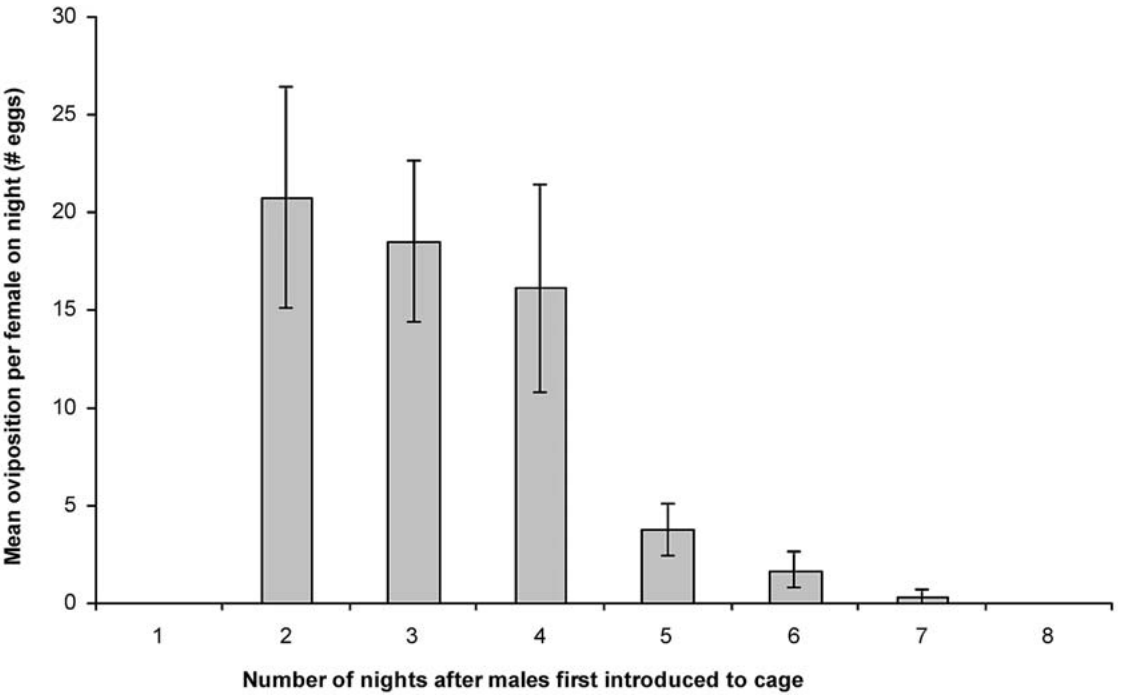

Fig. 2. Timing of oviposition by female *A. camptozonale* moths. Male moths were present in cages on the first night, but were removed early on the second d. Studies used 1 female moth per cage. Plant oviposition bouquets changed daily. Means based on observation of $n = 24$ female moths. Bars show standard errors.

Individual Male Studies

Male moths were never observed to mate with more than 1 female per night (Table 4). Mean number of lifetime matings per male was 2.0 ± 0.32, with a maximum of 3 lifetime matings and a minimum of 1 lifetime mating. Five male moths, paired with a total of 74 virgin females, only managed to mate with 10 of these females during their combined lifetimes. Mean longevity of male moths was 6.6 ± 1.2 d.

Discussion

Adult *A. camptozonale* emerged from pupae over a period of about 7 d from the day the first adult emerged, with peak emergence occurring on the second day after emergence began. Males and females emerged on every day of the emergence cycle, but the bulk of female emergence occurred earlier than for males, as has been documented for other lepidopteran species (Uematsu & Morikawa 1997). The laboratory colony of *A. camptozonale* was female-biased with an overall sex ratio of 1:1.3 (♂:♀).

In colony studies, significant differences were seen in the percentages of females that were mated at the 3 different sex ratios. A significantly higher percentage of females were mated at high male sex ratios (20♂:10♀) than were mated at lower male sex ratios (10♂:10♀ and 5♂:10♀). This same trend towards higher percentages of mated females in cages with high male sex ratios was visible in studies of individual females, although the trend was not statistically significant. No significant differences in spermatophore numbers were found in mated females across the different sex ratios, in either the colony or individual studies. This indicates that male sex ratio had an effect only on the proportion of females that mated within a cage and not on the number of times that each female mated. The vast majority of mated females in the colony studies (90%) and the individual female studies (96%), had mated only once. This finding together with the spermatophore data suggest that *A. camptozonale* females are likely to be functionally monandrous under field conditions, because mating frequencies observed under field conditions tend to be lower than those observed in the lab (Sadek 2001).

Cage sex ratio was found to have a significant effect on larval production in the colony studies. Mean larval production per cage at the 20♂:10♀ ratio was significantly higher than mean larval productions observed in cages at the 5♂:10♀ or 10♂:10♀ ratios. However, because there was no evidence that females in cages at the highest male sex ratio (20♂:10♀) mated a greater number of times than females maintained at lower male sex ratios, the observed increase in larval production must have been due to the higher percentage of females that mated and subsequently went on to oviposit in these cages.

In the individual female studies, high percentages of once-mated females were found at each of the 3 cage sex ratios. Because mating frequencies were essentially the same for females across each of these sex ratios, it was not surprising that cage sex ratio didn't explain significant amounts of the variation seen in mean larval production per female, mean female longevity or the timing of oviposition. Female longevity in these studies averaged 5.7 d. Daily oviposition data showed that females continued to lay eggs up until the day of their death, although 90% of eggs were laid between the second and fourth nights. These findings indicate that *A. camptozonale* females are relatively short lived compared with females of other lepidopteran species (Bento et al. 2006; Hughes et al. 2000; Svensson et al. 1998). In addition, the pre-ovipositional period was short and there was little if any post-ovipositional period. As such, the majority of the lifespan of female *A. camptozonale* is occupied directly with aspects of reproduction, and any events that delay mating or otherwise occupy her time, are likely to nega-

Table 4. Mating behavior of individual male moths supplied daily with excess virgin female moths.

	Number of female moths mated/number exposed to males on night[2]									
Male moth[1]	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6	Night 7	Night 8	Night 9	Night 10
#1	1/5	1/3	0/3	1/3	0/3	0/1	0/1	0/1	0/1	0/1
#2	1/5	1/3	0/3	0/3	0/3	—	—	—	—	—
#3	1/5	0/3	0/3	—	—	—	—	—	—	—
#4	1/5	1/3	0/3	0/3	0/3	0/1	—	—	—	—
#5	1/2	1/2	0/1	0/1	—	—	—	—	—	—

[1]Data based on multi-night observations of 5 male moths.

[2]Virgin female moths added daily into cages with each male moth. Females recaptured and dissected the next day to see whether they had mated overnight (indicated by presence of spermatophores in the bursa copulatrix).

tively impact her reproductive output. This may explain why *A. camptozonale* recapture rates were low and no evidence of field establishment was found following field releases of moths in 2005. Stresses on newly emerged moths associated with collection and transportation to release sites at a critical time in the life cycle when they would ordinarily be focused on mating and oviposition, could easily have a negative impact on moth reproduction in the field and reduce the likelihood of successful establishment.

Austromusotima camptozonale females seldom (1 of 29 females) mated more than once per night even when they were confined with an excess of virgin male moths. Similarly, virgin male moths were never observed to mate with more than 1 female a night, even when surplus virgin female moths were present. This pattern of behavior in *A. camptozonale* is consistent with the general pattern observed in Lepidoptera, where male and female moths typically mate only once per night (Gadenne et al. 2001). Reductions in female receptivity following mating are associated with male factors transferred during copulation (Raabe 1986; Foster & Ayers 1996), and are temporary for polyandrous species, or permanent for monandrous species (Gadenne et al. 2001). The low propensity of males to mate more than once per night appears to be due to energetic and time constraints associated with replacement of a large spermatophore (Hughes et al. 2000).

In conclusion, female *A. camptozonale* moths are short-lived and heavily preoccupied with oviposition during their adult lifespan. As such, adult *A. camptozonale* may not be the best life stage to use in a biological control release program, and future field colonization efforts will focus on larval releases. Released larvae will be able to feed and pupate in the field, and emerging female moths will be free to focus on mating and oviposition without enduring the stresses associated with handling and transportation to field sites. Meanwhile, male-biased sex ratios were shown to increase larval production in laboratory colonies by reducing the percentage of unmated female moths. By shedding light on these 2 important aspects of the reproductive biology of *A. camptozonale* the research detailed herein suggests a strategy for maximizing colony production and suggests a possible approach for improving *A. camptozonale* field colonization efforts.

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