

Delayed mating in tortricid leafroller species: simultaneously aging both sexes prior to mating is more detrimental to female reproductive potential than aging either sex alone

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Abstract

The effect of delayed mating on reproductive potential, longevity and oviposition period of female redbanded leafroller, *Argyrotaenia velutinana* (Walker) and Pandemis leafroller, *Pandemis pyrusana* Kearfott, was investigated in the laboratory. Virgin female or male moths of each species were held for 1, 2, 4, 6 or 10 days prior to pairing with one-day-old virgin conspecifics of the opposite sex. In addition, reproductive potential was assessed when both sexes of each species were aged for those periods prior to pairing. The expected reproduction of female *A. velutinana* was reduced by 34, 53, 71 and 81% for 2, 4, 6 and 10-day delays in female mating, respectively. For *P. pyrusana*, expected reproduction was reduced by 47, 74, 85 and 93% for 2, 4, 6 and 10-day delays in female mating, respectively. Increasing male age at mating in both species had a lesser effect on female reproductive output compared with increasing female age at mating. As male *A. velutinana* age at mating increased, the expected reproduction of female *A. velutinana* was reduced by 15, 45, 54 and 70% for 2, 4, 6 and 10-day delays, respectively. Comparing male *P. pyrusana* of various ages at mating, expected reproduction was reduced by 14, 42, 64 and 79% for 2, 4, 6 and 10-day delays in mating, respectively. The decrease in female reproduction when both sexes were aged prior to mating was higher than when either sex alone was aged prior to pairing with a one-day-old virgin of the opposite sex. The expected reproduction of female *A. velutinana* was reduced by 60, 83, 96 and 98% for 2, 4, 6 and 10-day delays in mating of both sexes, respectively. Only 7.5% of female eggs hatched when both sexes of *A. velutinana* were aged ten days prior to mating. When simultaneously aging both sexes of *P. pyrusana* prior to mating, expected reproduction was reduced by 71, 93, 96 and 99% for 2, 4, 6 and 10-day delays in mating, respectively. No *P. pyrusana* eggs hatched after a ten-day delay of mating for both sexes. For both species, female longevity increased and duration of oviposition period decreased with increasing female age at mating. Our results demonstrate that delayed mating in both females and males negatively affects

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female reproductive output in both species and that simultaneous aging of both sexes prior to mating has a greater effect than aging either sex alone. Our results suggest that laboratory studies that have paired aged females or aged males with conspecifics of optimal reproductive maturity have likely underestimated the effects of delayed mating on reproductive output.

Keywords: *Argyrotaenia velutinana*, Redbanded leafroller, *Pandemis pyrusana*, Pandemis leafroller, mating disruption, reproductive potential, delayed mating

(Accepted 18 June 2008)

Introduction

Female age at mating has been shown to affect the reproductive potential of several lepidopteran species. An effect of 'delayed mating', or greater age at first mating, is reduced reproductive potential compared with younger females (e.g. Barrer, 1976; Proshold *et al.*, 1982; Torres-Vila *et al.*, 2002). Increased age of virgin females at mating has been shown specifically to decrease female fertility (e.g. Kiritani & Kanoh, 1984; Lingren *et al.*, 1988; Proshold, 1996; Knight, 1997) and/or fecundity (e.g. Ellis & Steele, 1982; Lingren *et al.*, 1988; Walker, 1991; Karalius & Buda, 1995; Knight, 1997). For example, the decreases in female moth fecundity and/or fertility occurring from delayed mating have resulted from: deposition of unfertilized eggs by virgin females prior to mating (Foster *et al.*, 1995); decreased availability or reabsorbed nutrients from maturing ova with increasing age (Proshold, 1996); decreased ability to maintain and utilize viable sperm with increasing age (Proshold, 1996); buildup of oocyte degradation products interfering with sperm transfer (Torres-Vila *et al.*, 2002); or various combinations of the above factors. Similar, but less pronounced, effects of delayed mating have also been demonstrated in a coleopteran (Wenninger & Averill, 2006).

Fewer investigations have focused on the effect of male age and mating history on female reproductive potential; however, pairing older males with virgin females has also been shown to reduce female lifetime fecundity (e.g. Jiménez-Pérez & Wang, 2003). Some have reported that lifetime fecundity is lower for female Lepidoptera that mate with previously-mated, compared with virgin, males (Rutowski *et al.*, 1987; Mbata & Ramaswamy, 1990; Jiménez-Pérez & Wang, 2004), whereas others have not (Ward & Landolt, 1995; Sadek, 2001). Despite considerable evidence that both female and male age affect female reproductive performance, the interaction between female and male delayed mating has received little attention. To our knowledge, only one study has investigated the interactive effect of aging both sexes prior to mating (Jiménez-Pérez & Wang, 2003), showing an additive decrease in female reproductive potential when both sexes were aged.

The detrimental effects of delayed mating on female reproductive output is thought to significantly contribute to population control by mating disruption in cases where mate-finding is not completely prevented (Jones & Aihara-Sasaki, 2001). There are several examples where population control and crop protection were successfully achieved by deployment of synthetic pheromones, despite only moderate levels of mating disruption (McLaughlin *et al.*, 1994; Cardé & Minks, 1995; Knight, 1996; Calkins, 1998; Angeli *et al.*, 1999). Consequently, delaying mating of female moths of various

species by 2–7 days is thought to significantly improve the efficacy of mating disruption (e.g. Knight, 1997; Torres-Vila *et al.*, 2002). Although the vast majority of research on delayed mating has been conducted in the laboratory, field evidence also suggests that pest control by mating 'disruption' may be due, in large part, to the effects of a mating 'delay' (Knight, 1997). Furthermore, life table analyses have shown that mating delay and the effect of natural enemies on population suppression should act synergistically (Jones & Aihara-Sasaki, 2001). This should be especially important in orchards treated with pheromones, given that decreased input of pesticides under mating disruption should lead to higher population densities of biological control agents (Epstein *et al.*, 2000).

Fraser & Trimble (2001) investigated the effect of delayed mating by pairing females of various ages with 2–3-day-old virgin males in the oriental fruit moth, *Grapholita molesta* (Busck). They found that reproductive output declined by only 7% for each day mating was delayed post emergence. Based on this result, it was concluded that effective control of *G. molesta* with pheromones was more likely to be achieved by completely preventing mating rather than by delaying mating. However, investigating the effect of delayed mating by aging females only and not considering the additional effect of aging males may give an inaccurate estimate of the degree to which delayed mating impacts female reproductive output and the potential efficacy of pheromone-based disruption. Disruption should delay mating not only of stationary calling virgin females but also of searching males in the face of many competing point sources of synthetic pheromone (Stelinski *et al.*, 2004). This is particularly true given the protandrous male emergence common in most moth species (McNeil, 1991) and the fact that disruption products are typically deployed before the emergence of male moth flight (Stelinski *et al.*, 2007). Thus, the effect of male age at mating should also be considered when determining the potential effects of delayed mating on female reproductive output.

The objectives of the current investigation were to determine the effect of age at mating of both females and males of two leafroller species (redbanded leafroller, *Argyrotaenia velutinana* (Walker) and Pandemis leafroller, *Pandemis pyrusana* Kearfott) on female fertility, fecundity, longevity, oviposition period and egg fertility.

Materials and methods

Insect cultures

The *A. velutinana* culture originated from a long-established laboratory colony maintained at Geneva, NY,

USA (by W. Roelofs). The *P. pyrusana* culture originated from a laboratory colony maintained at Wenatchee, WA, USA (by J. Brunner) and that had been started from specimens obtained from a commercial apple orchard in Yakima, WA, USA. Additions of wild *P. pyrusana* collected from an untreated apple orchard in Wenatchee, WA were added to this colony in 2000 and 2003. Both species were reared year-round, without diapause at 24°C and 60% RH, on a pinto bean-based diet (Shorey & Hale, 1965) under a 16:8 (L:D) photoperiod. Pupae of each species were segregated by sex and maintained in one-liter plastic cages with a 5% sucrose solution food source for emerged adults prior to experiments. For all experiments, the weight of pupae used was within a 2.5-mg range according to the protocol of Fraser & Trimble (2001) to minimize the effect of moth size on fecundity. For *A. velutinana*, the mean \pm SE male and female pupal weight (range) was 12.80 \pm 2.82 mg (11.55–14.05) and 20.25 \pm 2.2 mg (19.00–21.50), respectively. For *P. pyrusana*, the mean pupal weight (range) for males and females was 27.65 \pm 1.87 mg (26.4–28.9) and 55.75 \pm 2.35 mg (54.50–57.00), respectively.

Effect of female, male or female and male age at mating on reproductive performance

In the first experiment, we tested the effect of female age at mating on female fertility (percentage of females laying fertile eggs), fecundity (total number of eggs laid), percent egg fertility (percent developing to the blackhead stage regardless of whether the larvae hatched) and percent eggs hatching. Virgin female moths were held for 1, 2, 4, 6 or 10 days prior to pairing with one-day-old virgin males. Single mating pairs were established in one-liter plastic translucent cages lined with wax paper and provided with sugar water as described in Gökçe *et al.* (2007). Females were randomly assigned to an aging treatment at emergence. Five females were paired with five males as individual pairs for each treatment on eight separate replicate days, which were considered as blocks. Cages containing moths were maintained for 22 days after introduction of mating pairs. The wax paper lining of the plastic cages was removed and replaced daily. Individual eggs within egg masses were counted for the duration of the experiment or until female moths died. Egg masses on removed wax paper were maintained for 15 days and examined daily for egg development (blackhead stage) or hatching. The experiment was conducted separately for each species. Female moths of both species were dissected following both experiments to inspect their bursa copulatrix for presence of a spermatophore, confirming mating status. Experiments were conducted under the temperature and light cycle conditions described earlier for rearing. Two subsequent experiments were conducted identically to the first except that, in experiment 2, males were aged for the above-described durations prior to pairing with one-day-old virgin females and, in experiment 3, both sexes of each species were simultaneously aged for those durations prior to pairing. In the latter case, for each treatment, females were always paired with males of the same age.

Effect of female age at pairing on female longevity and oviposition period

In a separate experiment, females were paired 1, 2, 4, 6 or 10 days after emergence with one-day-old virgin males or

left unpaired and unmated in one-liter plastic cages lined with wax paper as described above. Females were maintained in cages until death (max. 28 days); wax paper lining was replaced daily. Female longevity and oviposition period (number of days between onset and termination of egg laying) were recorded. Females were assigned to an aging treatment at emergence and 30 pairs were established for each aging period. The experiment was conducted separately for each species.

Statistical analyses and other calculations

Percentages of female fertility, fecundity, egg fertility and eggs hatching were arcsine square root transformed (to normalize the distributions and homogenize variance) and then subjected to a randomized complete block (blocked by day) analysis of variance (ANOVA). Data for total number of eggs hatching, female longevity and oviposition period were $\ln(x+1)$ transformed prior to ANOVA. Differences in pairs of means were separated using Fisher's protected least significant difference tests (SAS Institute, 2000). Analysis of covariance was used to determine whether pupal weight had an effect on female fertility or fecundity and egg fertility (SAS Institute, 2000). In all cases, the significance level was $\alpha < 0.05$. Expected and relative expected reproduction was calculated according to Kiritani & Kanoh (1984) and Fraser & Trimble (2001). Expected reproduction for an xth-day-old female was calculated as: xth day survival rate \times percent successful mating \times total viable eggs. Female death prior to pairing with males did not occur; and, therefore, the adjustment for female survival rate was not necessary. Relative expected reproduction for an xth-day-old female was calculated as: expected reproduction of an xth-day-old female per expected reproduction maximum, or the highest reproduction obtained for a female xdays old.

Results

Effect of male and female pupal weight

Variation in female pupal weight significantly affected fecundity for both *A. velutinana* ($N = 30$ per sex) ($F_{4,144} = 23.6$, $P < 0.001$) and *P. pyrusana* ($N = 30$ per sex) ($F_{4,144} = 31.1$, $P < 0.001$). However, female pupal weight had no significant effect on egg fertility ($F_{s4,144} = 2.1$ and 0.03, $P_s = 0.1$ and 0.8), female longevity ($F_{s4,144} = 0.6$ and 0.05, $P_s = 0.2$ and 0.8) or oviposition period ($F_{s4,144} = 2.6$ and 1.1, $P_s = 0.1$) in either species. Male pupal weight had no significant effect on female fecundity ($F_{s4,144} = 0.3$ and 0.1, $P_s = 0.5$ and 0.1), egg fertility ($F_{s4,144} = 0.02$ and 0.4, $P_s = 0.7$ and 0.1), female longevity ($F_{s4,144} = 0.06$ and 1.5, $P_s = 0.7$ and 0.2) or oviposition period ($F_{s4,144} = 2.3$ and 1.1, $P_s = 0.1$) for *A. velutinana* or *P. pyrusana*, respectively.

Effect of female age when mated on reproductive potential

For *A. velutinana*, the percentage of females laying fertile eggs ($F_{4,35} = 27.3$, $P < 0.001$), total number of eggs laid ($F_{4,35} = 16.2$, $P = 0.002$), egg fertility ($F_{4,35} = 32.1$, $P < 0.001$) and percentage of eggs hatching ($F_{4,35} = 39.2$, $P < 0.001$) significantly decreased as mating was delayed from one to ten days (table 1). The expected reproduction of female *A. velutinana* was reduced by 34, 53, 71 and 81% for 2, 4, 6 and 10-day delays in female mating, respectively (table 1).

Table 1. Mean \pm SEM female fertility, fecundity, egg fertility, % eggs hatching and expected reproduction of female *A. velutinana* and *P. pyrusana* when one-day-old males were paired with females of various age post eclosion.

Species	Female age when mated (days)	Percentage of females laying fertile eggs	Total number of eggs laid per five females	% Egg fertility	% Eggs hatching	Expected ¹ reproduction	Relative ² expected reproduction
<i>A. velutinana</i>	1	97.5 \pm 1.3a	120 \pm 15.1a	91.0 \pm 11.6a	80.5 \pm 8.5a	106	100
<i>A. velutinana</i>	2	92.5 \pm 1.8a	90.6 \pm 32.0b	83.0 \pm 8.5b	71.5 \pm 11.9a	70	66
<i>A. velutinana</i>	4	85.0 \pm 2.5b	76.0 \pm 11.9c	77.1 \pm 8.9b	69.9 \pm 8.6b	50	47
<i>A. velutinana</i>	6	75.0 \pm 3.7c	62.4 \pm 49.3c	66.3 \pm 7.5c	66.3 \pm 7.6b	31	29
<i>A. velutinana</i>	10	70.7 \pm 2.5c	49.3 \pm 11.0d	49.5 \pm 6.3d	49.5 \pm 6.3c	17	16
<i>P. pyrusana</i>	1	100.0 \pm 0.0a	402.4 \pm 60.7a	93.8 \pm 14.8a	88.3 \pm 6.7a	377	100
<i>P. pyrusana</i>	2	90.0 \pm 1.9ab	256.0 \pm 44.4b	87.2 \pm 10.7a	81.0 \pm 10.6a	201	53
<i>P. pyrusana</i>	4	82.5 \pm 2.3b	166.3 \pm 32.5c	71.0 \pm 6.2b	71.4 \pm 6.2b	97	26
<i>P. pyrusana</i>	6	70.0 \pm 3.3c	138.8 \pm 21.9cd	56.2 \pm 4.7c	56.2 \pm 5.0c	55	15
<i>P. pyrusana</i>	10	57.5 \pm 2.3d	105.6 \pm 22.2d	45.8 \pm 2.6c	45.8 \pm 2.6c	28	7

Means in a column (within species) followed by the same letter are not significantly different (ANOVA followed by LSD test, $P > 0.05$).

¹ Expected reproduction was calculated as: xth day survival rate \times percent successful mating \times total viable eggs.

² Relative expected reproduction was calculated as: expected reproduction of an xth-day-old female/expected reproduction max. or the highest reproduction obtained for a female xdays old.

Table 2. Mean \pm SEM female fertility, fecundity, egg fertility, % eggs hatching and expected reproduction of female *A. velutinana* and *P. pyrusana* when one-day-old females were paired with males of various age post eclosion.

Species	Male age when mated (days)	Percentage of females laying fertile eggs	Total number of eggs laid per five females	% Egg fertility	% Eggs hatching	Expected ¹ reproduction	Relative ² expected reproduction
<i>A. velutinana</i>	1	100.0 \pm 0.0a	153.1 \pm 35.1a	91.7 \pm 10.5a	79.4 \pm 9.0a	140	100
<i>A. velutinana</i>	2	97.5 \pm 1.3a	136.0 \pm 28.7a	90.1 \pm 11.2a	76.6 \pm 5.7a	119	85
<i>A. velutinana</i>	4	85.0 \pm 2.5b	108.5 \pm 21.0ab	83.6 \pm 7.6ab	72.4 \pm 5.6a	77	55
<i>A. velutinana</i>	6	82.5 \pm 3.0b	98.1 \pm 20.0bc	79.6 \pm 4.1bc	69.5 \pm 5.7ab	64	46
<i>A. velutinana</i>	10	70.7 \pm 2.5c	86.6 \pm 20.3c	69.1 \pm 5.5c	59.6 \pm 4.4b	42	30
<i>P. pyrusana</i>	1	100.0 \pm 0.0a	470.6 \pm 59.7a	91.4 \pm 11.3a	82.3 \pm 4.3a	430	100
<i>P. pyrusana</i>	2	97.5 \pm 1.3a	418.1 \pm 47.9ab	90.4 \pm 10.4a	77.1 \pm 3.8a	369	86
<i>P. pyrusana</i>	4	82.5 \pm 3.0b	365.1 \pm 41.5b	82.5 \pm 6.9a	74.8 \pm 4.0a	249	58
<i>P. pyrusana</i>	6	75.0 \pm 3.7c	304.8 \pm 29.6b	67.9 \pm 6.2b	68.4 \pm 5.8ab	155	36
<i>P. pyrusana</i>	10	72.5 \pm 3.8c	214.8 \pm 31.7c	57.6 \pm 9.3b	54.9 \pm 1.7b	90	21

Means in a column (within species) followed by the same letter are not significantly different (ANOVA followed by LSD test, $P > 0.05$).

¹ Expected reproduction was calculated as: xth day survival rate \times percent successful mating \times total viable eggs.

² Relative expected reproduction was calculated as: expected reproduction of an xth-day-old female/expected reproduction max. or the highest reproduction obtained for a female xdays old.

For female *P. pyrusana*, there was also a significant decrease in the percentage of females laying fertile eggs ($F_{4,35} = 19.0$, $P = 0.01$), total number of eggs laid ($F_{4,35} = 21.5$, $P < 0.001$), egg fertility ($F_{4,35} = 44.1$, $P < 0.001$) and percentage of eggs hatching ($F_{4,35} = 14.1$, $P = 0.02$) as mating was delayed (table 1). For this species, expected reproduction was reduced by 47, 74, 85 and 93% for 2, 4, 6 and 10-day delays in female mating, respectively (table 1).

effect on female reproductive output compared with increasing female age at mating. As male *A. velutinana* age at mating increased, the expected reproduction of female *A. velutinana* was reduced by 15, 45, 54 and 70% for 2, 4, 6 and 10-day delays, respectively (table 2). For male *P. pyrusana* of various age at mating, expected reproduction was reduced by 14, 42, 64 and 79% for 2, 4, 6 and 10-day delays in mating, respectively (table 2).

Effect of male age when mated on reproductive potential

As observed with females, increasing male age at mating significantly decreased the percentage of females laying fertile eggs ($F_{s4,35} = 17.4$ and 22.1, $P_s = 0.01$ and 0.004), total number of eggs laid ($F_{s4,35} = 10.7$ and 14.2, $P_s = 0.04$ and 0.02), egg fertility ($F_{s4,35} = 19.8$ and 22.4, $P_s = 0.01$ and < 0.001) and percentage of eggs hatching ($F_{s4,35} = 28.2$ and 18.6, $P_s < 0.001$) for *A. velutinana* and *P. pyrusana*, respectively (table 2). Increasing male age at mating in both species had a lesser

Effect of male and female age when mated on reproductive potential

Simultaneously aging both females and males prior to mating significantly decreased the percentage of females laying fertile eggs ($F_{s4,35} = 31.9$ and 19.3, $P_s < 0.001$), total number of eggs laid ($F_{s4,35} = 16.3$ and 11.4, $P_s = 0.01$ and 0.05), egg fertility ($F_{s4,35} = 10.3$ and 16.3, $P_s = 0.01$) and percentage of eggs hatching ($F_{s4,35} = 35.8$ and 16.2, $P < 0.001$ and $P = 0.01$) for *A. velutinana* and *P. pyrusana*, respectively (table 3). The

Table 3. Mean \pm SEM female fertility, fecundity, egg fertility, % eggs hatching and expected reproduction of female *A. velutinana* and *P. pyrusana* when females of various age were paired with males of equivalent age post eclosion.

Species	Male and female age when mated (days)	Percentage of females laying fertile eggs	Total number of eggs laid per five females	% Egg fertility	% Eggs hatching	Expected ¹ reproduction	Relative ² expected reproduction
<i>A. velutinana</i>	1	95.0 \pm 1.6a	135.1 \pm 19.1a	93.5 \pm 13.8a	76.9 \pm 7.8a	120	100
<i>A. velutinana</i>	2	90.0 \pm 1.9a	88.5 \pm 9.1b	60.2 \pm 7.3b	51.3 \pm 6.1b	48	40
<i>A. velutinana</i>	4	65.0 \pm 3.1b	57.5 \pm 9.3c	54.1 \pm 9.0b	39.8 \pm 4.7c	20	17
<i>A. velutinana</i>	6	57.3 \pm 3.0b	26.6 \pm 6.4d	32.7 \pm 5.8c	17.3 \pm 2.9d	5	4
<i>A. velutinana</i>	10	45.0 \pm 2.5c	21.9 \pm 6.1d	17.5 \pm 3.2d	7.5 \pm 1.9e	2	2
<i>P. pyrusana</i>	1	100.0 \pm 0.0a	427.1 \pm 38.4a	94.2 \pm 8.5a	72.7 \pm 4.1a	402	100
<i>P. pyrusana</i>	2	92.5 \pm 1.8a	188.5 \pm 26.8b	67.9 \pm 4.7b	54.9 \pm 5.0b	118	29
<i>P. pyrusana</i>	4	57.5 \pm 2.3b	105.8 \pm 12.1c	48.1 \pm 5.3cd	31.6 \pm 4.0c	29	7
<i>P. pyrusana</i>	6	55.0 \pm 3.7b	80.1 \pm 11.0d	36.8 \pm 3.7d	13.0 \pm 2.1d	16	4
<i>P. pyrusana</i>	10	37.5 \pm 4.0c	39.9 \pm 8.3e	18.1 \pm 3.3e	0.0 \pm 0.0e	3	1

Means in a column (within species) followed by the same letter are not significantly different (ANOVA followed by LSD test, $P > 0.05$).

¹ Expected reproduction was calculated as: xth day survival rate \times percent successful mating \times total viable eggs.

² Relative expected reproduction was calculated as: expected reproduction of an xth-day-old female/expected reproduction max. or the highest reproduction obtained for a female xdays old.

decrease in female reproduction when both sexes were aged prior to mating was higher than when either sex alone was aged prior to pairing with a one-day-old virgin of the opposite sex. The expected reproduction of female *A. velutinana* was reduced by 60, 83, 96 and 98% for 2, 4, 6 and 10-day delays in mating of both sexes, respectively (table 3). Only 7.5% of female eggs hatched when both sexes of *A. velutinana* were aged ten days prior to mating (table 3). When simultaneously aging both sexes of *P. pyrusana* prior to mating, expected reproduction was reduced by 71, 93, 96 and 99% for 2, 4, 6 and 10-day delays in mating, respectively (table 3). Of the 18% of viable eggs deposited by female *P. pyrusana* when both sexes were aged ten days prior to mating, none hatched (table 3).

Effect of female age when mated on longevity and reproductive potential

Longevity of virgin females ($F_{5,174} = 14.2$, $P = 0.04$) and duration of oviposition period ($F_{5,174} = 33.1$, $P < 0.001$) were significantly longer compared with one-day-old females paired with one-day-old *A. velutinana* males (table 4). Female longevity was significantly longer ($F_{5,174} = 18.6$, $P = 0.01$), and the duration of the oviposition period was significantly shorter ($F_{5,174} = 14.2$, $P = 0.05$) for female *A. velutinana* paired six and ten days post emergence with one-day-old males than with females paired one day post emergence (table 4). When paired with one-day-old male *P. pyrusana*, female longevity ($F_{5,174} = 18.2$, $P = 0.02$) was significantly longer for virgin females than with females paired at all ages tested post emergence; however, the duration of the oviposition period ($F_{5,174} = 16.0$, $P = 0.05$) was not significantly different except when females were mated at 4, 6 or 10 days of age when it was significantly shorter (table 4). Longevity ($F_{5,174} = 11.2$, $P = 0.05$) was significantly longer for female *P. pyrusana* paired ten days after emergence with one-day-old males compared with females paired 1, 2, 4 and 6 days after emergence. However, four-, six- and ten-day-old female *P. pyrusana* had a significantly shorter oviposition period ($F_{5,174} = 12.7$, $P = 0.04$) than one-day-old females when both treatments were paired with one-day-old males (table 4).

Table 4. Mean \pm SEM longevity and duration of oviposition of female *A. velutinana* and *P. pyrusana* when females of various age post eclosion were paired with one-day-old males.

Species	Female age when mated (days)	Female longevity (days)	Oviposition period (days)
<i>A. velutinana</i>	1	11.1 \pm 0.4a	7.1 \pm 0.3a
<i>A. velutinana</i>	2	10.8 \pm 0.4a	7.3 \pm 0.3a
<i>A. velutinana</i>	4	11.5 \pm 0.5a	6.6 \pm 0.3ab
<i>A. velutinana</i>	6	14.1 \pm 0.4b	5.7 \pm 0.5b
<i>A. velutinana</i>	10	15.6 \pm 0.5b	3.9 \pm 0.3c
<i>A. velutinana</i>	Virgin	20.7 \pm 0.7c	10.9 \pm 0.5d
<i>P. pyrusana</i>	1	10.2 \pm 0.4a	7.6 \pm 0.4a
<i>P. pyrusana</i>	2	10.1 \pm 0.4a	6.7 \pm 0.4ab
<i>P. pyrusana</i>	4	10.9 \pm 0.5a	4.9 \pm 0.4b
<i>P. pyrusana</i>	6	12.3 \pm 0.6a	4.3 \pm 0.3bc
<i>P. pyrusana</i>	10	15.8 \pm 0.6b	3.6 \pm 0.2d
<i>P. pyrusana</i>	Virgin	17.8 \pm 0.5c	8.2 \pm 0.5a

Means in a column (within species) followed by the same letter are not significantly different (ANOVA followed by LSD test, $P > 0.05$).

Discussion

Delayed mating reduced the reproductive potential of both female *A. velutinana* and *P. pyrusana*, two economically important tree fruit pest species (Knight & Turner, 1999; Stelinski *et al.*, 2005), in which delayed mating has not been previously investigated. The effect of increased female age at mating on the reproductive output was more than twice as great as increased male age at mating for both species for a two-day delay of mating. For *A. velutinana*, expected reproduction decreased by 34% when females were aged two days prior to mating, but only 15% when males were aged two days prior to mating. The pattern was similar in *P. pyrusana*; there was a 47% reduction in expected reproduction when females were aged two days but only a 14% reduction when males were aged two days prior to mating. As mating of either sex was delayed more than two days, the effect of male age on reproductive output became more similar to that of females. A ten-day delay of female

and male mating resulted in 84 and 70% reductions in expected reproduction, respectively, in *A. velutinana* and 93 and 79% reductions, respectively, in *P. pyrusana*. Our results regarding the effect of increasing female age at mating on reproductive output are similar to other studies in which 2–3-day delays typically result in 40–50% reductions in female output (Leather *et al.*, 1985; van der Kraan & van der Straten, 1988; Proshold, 1996; Knight, 1997). However, our current data also point to the importance of increased male age at mating on female reproductive output, which also had a large impact on fecundity and fertility, particularly for virgin males four or more days of age. These results suggest that, as male moths age prior to mating, their ability to copulate with females or transfer sperm may decrease or sperm quality may be negatively affected.

As has been observed in previous studies (e.g. Proshold *et al.*, 1982; Kiritani & Kanoh, 1984; Karalius & Buda, 1995; Lingren *et al.*, 1988; Fraser & Trimble, 2001), delaying mating in females increased adult longevity maximally by 4–5 day. It is thought that allocation of nutritional reserves for egg maturation directly after mating may be responsible for decreasing lifespan of mated vs. virgin females (Proshold *et al.*, 1982). Alternatively, reabsorbing nutrients by virgin females experiencing delayed mating may prolong lifespan while decreasing lifetime fecundity (Wenninger & Averill, 2006). Lifespan of *A. velutinana* increased after a six-day delay of mating, while an increase in lifespan for *P. pyrusana* was only observed after a ten-day delay, suggesting that allocation of nutritional resources toward egg development may be greater in the former species compared with the latter.

The duration of the oviposition period was shorter in one-day-old mated *A. velutinana* compared with virgin moths, but not so in *P. pyrusana* (table 4), also suggesting greater nutritional resource allocation toward maturation of fertile eggs (Proshold *et al.*, 1982) in the former species compared with the latter. The duration of the oviposition period declined in *A. velutinana* after a six-day delay of mating but did so in *P. pyrusana* females after only a four-day delay. These data provide further support for the hypothesis that overall nutritional reserve allocation per female toward egg maturation may be greater in *A. velutinana* than in *P. pyrusana* given that mated females of the former species maintain a maximal oviposition period longer than females of the latter species. However, the duration of the oviposition period decreased overall with increasing mating delay in both species, which is consistent with previous studies (e.g. Spurgeon *et al.*, 1997; Fraser & Trimble, 2001; Torres-Vila *et al.*, 2002).

The majority of studies investigating delayed mating have paired females of various ages with males of a fixed, optimal age (see examples in table 1 of Wenninger & Averill (2006)). Although the effect of male age on female fecundity has been addressed in a few studies, the interaction of male and female age has received little attention (but see Jiménez-Pérez & Wang, 2003). Thus, the impact of delayed mating on female reproductive potential and its consequences under mating disruption have likely been underestimated in many species (e.g. *G. molesta*: Fraser & Trimble, 2001). In the current investigation, there appeared to be an additive effect of aging both sexes on the female expected reproduction in both species. Pairing four-day-old males and females reduced expected reproduction by 83 and 93% for *A. velutinana* and *P. pyrusana*, respectively, compared with

one-day-old moths. A six or more day delay of mating for both species nearly eliminated expected reproduction. These results underscore the importance of male age in addition to female age on reproductive potential of tortricids and suggest that delayed mating due to treatment with pheromone-based mating disruption may have an even greater impact on population control than has been previously speculated (Jones & Aihara-Sasaki, 2001). In a future investigation, we plan on determining how delayed mating impacts males' ability to locate females and female choice of mating partners as well as the contribution of these factors to population control by mating disruption.

Acknowledgement

We thank Angel Hoyte, Krista Buehrer and Martin Villarreal for diligent maintenance of insect colonies. A previous version of the manuscript was improved by Drs B. Barrett (University of Missouri) and E. Wenninger (USDA-ARS, Fort Pierce).

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