

# Developmental and Behavioral Effects and Retention of Incremental Rates of Rubidium Fed to *Grapholita molesta* (Lepidoptera: Tortricidae) in Dietary Medium<sup>1</sup>

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**Abstract** The effects of rubidium chloride were characterized on immature and adult *Grapholita molesta* (Busck) in an effort to determine its suitability as a label to mark, release and recapture this species in field-based dispersal studies. Neonate *G. molesta* (Busck) larvae were fed lima bean-based diet enriched with 0, 600, 3,000 or 6,000 mg RbCl / L liquid diet (ppm). Both male and female adult *G. molesta* were successfully marked at the 3 highest concentrations. Increasing concentrations did not alter pupal mass or larva-to-adult development (first instar to adult death), but adult longevity decreased in response to increasing Rb concentrations in diet. All males and 83.3% of females reared on diet with 6,000 ppm RbCl retained a detectable Rb signature through 168 h post eclosion. Labeled males were more likely to exhibit stationary zig-zag flight prior to flying upwind to a sex pheromone lure in a wind tunnel. These experiments demonstrate that rubidium chloride may be used to label immature *G. molesta* for subsequent release and recapture without significant detrimental effects to the insects.

**Key words** oriental fruit moth, tree fruits, dispersal, developmental

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In perennial crops, advancements in using pheromones for areawide or farmscale insect pest management requires a working knowledge of pest dispersal, and several previous experiments have centered on dispersal phenomena of insects with economic importance in perennial crops. Methods for marking both immature and adult *Lymantria dispar* (L.) with rubidium were established to monitor its movement in forests (Fleischer et al. 1989, 1990). In the Canadian blueberry cropping system, Rb labeling has been used to define the range of blueberry leaftier (*Croesia curvalana* (Kearfoot)) pheromone traps to structure effective trapping regimes for this pest (Polavarapu et al. 1992). The oligophagous tortricid oriental fruit moth, *Grapholita molesta* (Busck), is suspected to migrate within and between its *Malus* and *Prunus* host crops. A better understanding of this insect's spatial dynamics in particular would be critical for optimizing the potential of pheromone-mediated management strategies in those crops (Hull et al. 2002).

Labeling of a tortricid insect with Rb incorporated into dietary media has been successful with the tufted apple bud moth (*Platynota idaeusalis* (Walker)) in pome fruit

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(Knight et al. 1989). These authors quantified the effects of Rb on *P. idaeusalis* maturation and reproductive biology. Their goal was to incorporate Rb into the insects' egg masses to track oviposition and thereby female movement among and between apple orchards. They concluded that the tufted apple bud moth could be fed up to 3,000 mg RbCl / L liquid diet without adverse effects on its physiology. Dispersal of female *P. idaeusalis* as indicated by the recovery of Rb-labeled eggs was then quantified in a Pennsylvania apple orchard (Knight et al. 1990).

Although Rb appears to affect developmental biology of insects in a dose-dependent fashion, and useful rates are often found that enable Rb to be used as a marker, insect physiology affects Rb retention. Rb levels in insect tissues may be reduced by absorption into ovaries and ova (Knight et al. 1989) or by feeding (Rhodes et al. 1998). The effects of ingested Rb on insect behavior has been documented in several previous studies. Although Rhodes et al. (1998) did not suggest why Rb-labeled southern pine beetles (*Dendroctonus frontalis* [Zimmerman]) fed untreated wood retained less Rb over time than unfed labeled beetles, Rb labeling did not affect normal *D. frontalis* behaviors such as gallery excavation or reproduction (Rhodes et al. 1998). Woods and Streett (1996a, b) showed that grasshopper feeding was not altered by RbCl incorporated into baits and conjectured that Rb may be selectively incorporated into the cuticle, thereby affecting Rb retention. *Aedes* spp. dispersal, as quantified by recovery of Rb-labeled eggs, was not affected by Rb (Liew and Curtis 2004). The retention of Rb over time and its effect on tortricid flight behavior is currently not well represented by the entomological literature. Our purpose was to determine whether Rb used as a marking agent adversely affects various parameters of male and female *G. molesta* biology and behavior.

## Materials and Methods

**Source of test insects.** Adult female and male *G. molesta* from a colony at The Pennsylvania State Fruit Research and Extension Center (hereafter PSU-FREC), Biglerville, PA, USA were used in all experiments. The insects were originally collected from infested peaches and apples in commercial orchards in Adams Co., PA, and had been reared in colony for at least 10 generations.

**Rb enrichment and effects on life stages.** Rubidium chloride salts at treatment levels of 0, 600, 3,000 or 6,000 mg RbCl / L liquid diet (ppm) were incorporated into lima bean-based diet (Knight et al. 1989). Approximately 5 ml of liquid diet was poured into the bottom of 22-ml plastic cups. After the diet cooled, 4 - 5 oriental fruit moth neonate larvae (< 24 h old) were placed into the cups and the cups were capped. The larvae completed their development at  $22.2 \pm 2^\circ\text{C}$  and approx. 72% RH under an 16:8 L:D photo regimen as maintained for all *G. molesta* colonies used for research at the PSU-FREC. Adult longevity (i.e., lifespan from pupal eclosion to adult death) and larva-to-adult development (i.e., lifespan from placement of larvae on diet to adult death) were recorded in days for each individual. The mass of individual pupae ( $n = 132$  females total across treatments;  $n = 114$  males total across treatments) were measured to  $\pm 0.1$  mg and  $\pm 0.5$  mm, respectively. Moths with Rb concentrations exceeding 3 standard deviations from the mean of the unlabeled controls were considered labeled (Stimmann 1974).

Spectrophotometer readings of ppm Rb content for the 8 sex/dosage categories were transformed with a double square-root function (to impart symmetry and stabilize variance). Differences in transformed Rb levels between sexes within doses were

analyzed as single-factor AOV with PROC GLM (SAS Institute, Cary, NC; Littell et al. 2002). Where heterogeneity of variance was found according to Levene's test for homogeneity of variance despite the transformations, Welch's AOV is reported (SAS Institute 2003). The effects of Rb on pupal mass; larva-to-adult developmental time; and adult longevity within each sex were analyzed with linear regression using PROC REG (SAS Institute 2003). All 3 raw developmental metrics were transformed with the natural logarithm (ln) to stabilize variance. The response variables were the transformed developmental metrics; the continuous explanatory variable was the incremental Rb dosage in the diet. All *P* values < 0.05 were considered statistically significant.

**Rb persistence in moth tissues.** Neonate (< 24 h old) larvae were isolated into individual 22-ml capped plastic cups having approx. 5 ml lima bean-based diet with 6,000 ppm RbCl. Pupae were separated by sex and upon eclosion moths were randomly assigned to treatment groups of 24 h (*n* = 39 females; *n* = 11 males); 96 h (*n* = 43 females; *n* = 21 males); or 168 h (*n* = 24 females; *n* = 24 males). Moths were killed in a freezer the final day of their respective time treatments. The Rb persistence study was analyzed as a linear regression model (PROC REG SAS Institute 2003). The response variable was spectrophotometer reading of Rb content in ppm transformed with the natural logarithm; the continuous explanatory variable was the time (h) following adult eclosion. All *P* values < 0.05 were considered statistically significant.

**Rb effects on male flight behavior.** Larvae were reared on lima bean-based diet with either 0 or 6,000 ppm RbCl. Male pupae were isolated into 22-ml plastic cups. Pupae completed development in a growth chamber with a 16:8 L:D light cycle in the same room as the wind tunnel. As the moths eclosed, they were grouped according to the date of emergence and provided water ad libitum through a cotton dental wick.

The flight bioassays were conducted in a windowless room with lamps fixed to the frame of an approx. 0.9 × 0.9 × 1.8 m wind tunnel, simulating evening light. The moths were removed from the growth chamber approx. 5 - 15 min prior to the beginning of the flight periods, which began 3 h before the onset of the scotophase in the growth chamber. A low-dose (0.011 mg) *G. molesta* pheromone lure [93% Z8 - 12:Ac; 6% E8 - 12:Ac; and 1% Z8 - 12:OH (Baker and Cardé 1979)] (Suterra, Inc., Bend, OR) was suspended from a ring-stand by a hook fashioned from a paperclip, at the upwind end of the tunnel. The low-dose lure was considered appropriate to prevent the wind tunnel from being saturated with pheromone and a commercial formulation was used to mimic what pest managers would use in the field. Male moths 2 - 5 d old were transferred to the downwind end of the tunnel individually in mesh conical release cages. The cages were set approx. 25.4 cm from the floor of the wind tunnel on a steel plate supported by a ringstand, and the aluminum screen removed to allow the moth free access to the tunnel environment. The temperature in the wind tunnel room during the 5-day experiment was 24°C; the wind velocity in the wind tunnel was 0.50 (±0.03) m/sec. Over the course of the experiment, the ratios of control:Rb treated moths flown per day were as follows: Day 1 (16:17); Day 2 (18:19); Day 3 (21:19); Day 4 (20:17); and Day 5 (14:18) for a total of *n* = 89 control and *n* = 90 Rb-treated moths.

After release, males were observed for 2 min for the 5 behaviors indicative of recognition of, and flight to, a point-source of pheromone: wing-fanning (first sign of pheromone detection); take-off (initial flight from the platform); lock-on (zig-zag flight whereas airborne without movement upwind); upwind flight (directed movement toward the source); and source contact (physical landing on the pheromone lure). Each

moth was removed after 2 min regardless of activity. Flight behaviors were scored as +/- (success/failure). Upon removal, the moths were immediately killed by freezing and prepared for Rb-content analysis.

The effect of Rb dietary enrichment on the total number of behaviors of the behavior sequence completed by individual moths in the wind tunnel was analyzed with log-linear analyses of  $2 \times 2$  contingency tables having Rb dosage as the column variable and occurrence of each behavior as the row variable (SAS Institute 2003, Stokes et al. 2000). Stratified analysis of Rb label on the occurrence of behavior according to the day of the experiment was accomplished with Mantel-Haenszel statistics in PROC FREQ (SAS Institute 2003, Stokes et al. 2000). Rb levels between the moths in the two Rb treatment-levels were compared with a Wilcoxon sign-rank test (PROC NPAR1WAY, SAS Institute 2003, Stokes et al. 2000). All *P* values < 0.05 were considered statistically significant.

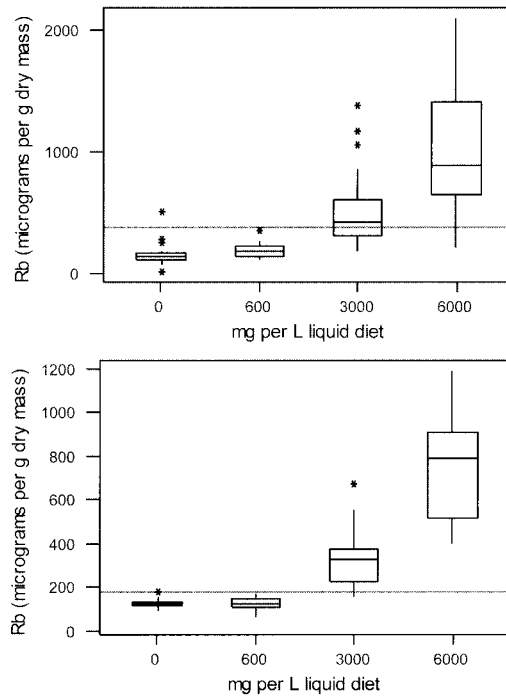
**Determination of Rb content.** In all experiments, Rb content is represented as  $\mu\text{g}$  Rb/g dry body mass. Moths were dried in a convection oven for 14 d and their dry masses measured ( $\pm 0.01$  mg). Digestion of the moths in the developmental and flight behavior studies were modified from Fleischer and Kirk (1994). The individual dry bodies were inserted into individual 15-ml screw-cap plastic test tubes and 0.3 ml of concentrated  $\text{HNO}_3$  added. The moths were then digested in a magnetron microwave (MDS-81D, CEM, Mathews, NC) set at  $674 \pm 21$  W for two cycles of 86 sec.

A technique modified from Van Steenwyk et al. (1978) was adopted after 2003 to digest moths analyzed from the persistence study. These moths were digested in 0.2 ml concentrated  $\text{HNO}_3$  in a convection oven for 14 d at  $60 \pm 5^\circ\text{C}$  rather than in the magnetron. This method permitted more samples to be digested simultaneously, thereby expediting the process of digestion. Upon cooling, the tubes were brought to 1 ml with 0.8 ml Na/L deionized water to prevent ionization of Rb. Upon completion of the digestions, the acid solution was vortexed for 10 sec and diluted to 1.0 ml with distilled water. The dilute acid solution was passed through a flame emission spectrophotometer (Perkin-Elmer 703, Wellesley, MA), set to detect Rb signatures (780 nm). All spectrophotometer analyses were conducted at the Materials Characterization Laboratory of The Pennsylvania State University Materials Research Institute (University Park, PA).

## Results

**Rb incorporation into tissues.** The Rb levels for unlabeled controls (mean + 3 SD) for female and male *G. molesta* were 377.9 and 181.2  $\mu\text{g}$  Rb/g dry mass, respectively (Fig. 1). Rb content in the 6,000-ppm treatments was significantly higher than in the readings from the 3,000-ppm treatments in both sexes (females: Wilcoxon *S* = 407;  $n_{3000} = 23$ ,  $n_{6000} = 29$ ; *P* = 0.0005; males: Wilcoxon *S* = 735;  $n_{3000} = 25$ ,  $n_{6000} = 21$ ; *P* < 0.0001), suggesting that the 6,000-ppm dosage would result in fewer samples below the established threshold than the 3,000-ppm dosage. At each level of enrichment above 0 ppm RbCl females incorporated significantly more Rb than males (0 ppm: Welch's *F* = 5.1; *df* = 1, 47.3; *P* = 0.029; 600 ppm: *F* = 42.7; *df* = 1, 64; *P* < 0.0001; 3,000 ppm: Welch's *F* = 9.1; *df* = 1, 35.8; *P* = 0.0046; 6,000 ppm: Welch's *F* = 4.1; *df* = 1, 44.7; *P* = 0.049) (Table 1).

**Rb effects on life stages.** Increasing Rb concentration had no significant effect on either female or male pupal mass or larva-to-adult development (Table 2). In both sexes, adult longevity decreased as Rb concentration in diet increased (Table 2).



**Fig. 1. Levels of rubidium found in adult female (top) and male (bottom) *G. molesta* after larval feeding on diet enriched with Rb. The horizontal line represents + 3 standard deviations from the mean rubidium content found in moths fed diet with 0 mg RbCl/L liquid diet (ppm).**

However, all parameter values were  $< 1 \times 10^{-5}$  indicating extremely small incremental changes to the response variables as the independent variables increased.

**Rb persistence in moth tissues.** At all times tested (24, 96 and 168 h) postadult eclosion, median Rb retention of larvae fed 6,000-ppm diet was above the established thresholds for both sexes (Fig. 2). Despite the significant loss of Rb over time, all male ( $n = 24$ ) and 83% of female ( $n = 24$ ) *G. molesta* still retained a label distinguishable from the baseline after 168 h (7 days) (Fig. 2). In the time between 96 and 168 h, female moths retained more Rb than males. In both males and females, Rb readings were higher 24 h following adult eclosion than at subsequent time periods and significant decreases in retention occurred over time (Table 3).

**Rb effects on male flight behavior.** Male moths reared on diet without RbCl had  $53.9 \pm 12.5$  (mean  $\pm$  SE)  $\mu\text{g}$  Rb/g dry mass in their tissues whereas moths reared on 6,000-ppm diet had significantly more Rb in their tissues ( $726.7 \pm 26.8$   $\mu\text{g}$  Rb/g dry mass) according to Wilcoxon sign-rank test analysis ( $S = 2340.0$ ;  $n_0 = 67$ ;  $n_{6000} = 82$ ;  $P < 0.0001$ ). Of the total moths ( $n_{\text{control}} = 89$ ;  $n_{\text{Rb}} = 90$ ) initially presented with a pheromone stimulus, the number of moths responding by wing-fanning did not differ significantly by Rb-treatment ( $G^2 = 3.61$ ;  $df = 1$ ;  $P = 0.06$ ) (Table 4). The percentage of moths exhibiting the wing-fanning behavior was high in both treatment groups (Table 4).

Table 1. Rubidium levels ( $\mu\text{g}$  /  $\text{mg}$  body mass) in male and female *G. molesta* mean ( $\pm$  SE).

Rb dose	Sex	n	Rb in tissues
0	Female	38	157.4 (11.9) a
	Male	28	126.6 (3.4) b
600	Female	34	188.2 (8.8) a
	Male	31	125.3 (4.6) b
3000	Female	23	533.8 (67.0) a
	Male	25	325.8 (25.1) b
6000	Female	29	1032.8 (95.5) a
	Male	21	749.7 (49.4) b

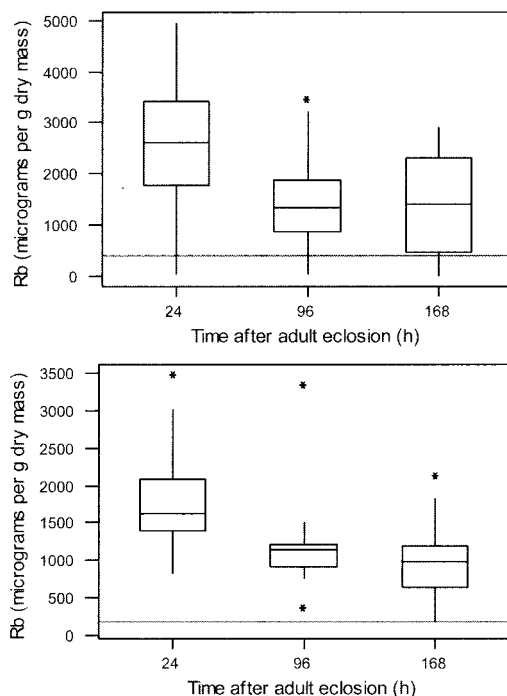
Means followed by different letters were significantly different in single factor analysis of variance of  $\sqrt{\text{transformed ppm values}}$  with (PROC GLM, Welch's F-statistic applied where appropriate according to Levene's test for homogeneity of variance, SAS Institute 2003).

Similar proportions of moths from each treatment exhibited take-off ( $G^2 = 0.43$ ;  $\text{df} = 1$ ;  $P = 0.51$ ) (Table 4). Following take-off, of the 63 unlabeled and 62 labeled moths taking flight, 23.8% and 41.9% from those respective treatments exhibited the locking-on behavior. The nearly 2-fold difference was significant ( $G^2 = 4.70$ ;  $\text{df} = 1$ ;  $P = 0.03$ ). Once achieving lock-on, however, all moths except one flew upwind ( $G^2 = 0.16$ ;  $\text{df} = 1$ ;  $P = 0.69$ ) and made source contact (Table 4).

Table 2. Effect of rubidium labeling ( $\text{mg}$  /  $\text{L}$  liquid diet) on *G. molesta* developmental parameters, mean ( $\pm$  SE).

Sex	Rb dose	n	Pupal mass ( $\text{mg}$ ) <sup>†</sup>	Adult longevity (d)	Larva-to-adult developmental time (d)
Female	0	41	13.1 (0.3) NS	20.9 (0.6) *	48.3 (0.7) NS
	600	35	12.5 (0.3)	21.1 (0.6)	48.2 (0.7)
	3000	26	12.7 (0.4)	21.0 (0.6)	48.5 (0.7)
	6000	30	12.4 (0.3)	18.8 (0.8)	47.0 (0.9)
Male	0	30	10.0 (0.2) NS	18.0 (0.8) *	44.3 (0.9) NS
	600	32	10.3 (0.3)	18.1 (0.8)	45.2 (0.9)
	3000	28	9.7 (0.2)	17.9 (1.0)	44.7 (1.0)
	6000	24	9.8 (0.3)	15.1 (1.0)	42.5 (1.1)

<sup>†</sup> Means of parameter measurements within sex in a column followed by "NS" were not significantly different at  $P = 0.05$  according to linear regression analyses. The asterisk indicates significance at  $P = 0.05$ . Comparisons were made between Rb doses separately within each sex. Response variables were transformed with the natural log ( $\ln$ ) function. Regressions expressing  $\ln(\text{adult longevity})$  as a function of Rb dose were:  $y = 3.0 - 0.00002x$ ;  $T = -2.49$ ;  $P = 0.01$ ;  $R^2 = 0.046$ ;  $\text{RMSE} = 0.22$  for females and  $y = 2.9 - 0.000036x$ ;  $T = -2.35$ ;  $P = 0.02$ ;  $R^2 = 0.047$ ;  $\text{RMSE} = 0.37$  for males.



**Fig. 2. Rb persistence at three time periods following adult eclosion in female (top) and male (bottom) *G. molesta* when fed diet incorporated with 6,000 mg RbCl/L liquid diet. The horizontal line represents + 3 SD from the mean of amount of rubidium found in moths fed diet with 0 mg RbCl/L liquid diet.**

On every day of the study, numerically more moths were observed wing-fanning than not wing-fanning, regardless of the treatment, although no significant statistical association between treatment and occurrence was found ( $Q_{MH} = 3.73$ ;  $df = 1$ ;  $P = 0.053$ ). Likewise, over the 5-day experiment, there was no date effect on take-off behavior ( $Q_{MH} = 0.47$ ;  $df = 1$ ;  $P = 0.49$ ). When the exhibition of locking-on behavior was analyzed controlling for the day of the release, the odds of unlabeled moths exhibiting lock-on were 0.53; that is, the unlabeled moths were 47% less likely to exhibit the behavior than the labeled moths. This odds ratio was significant ( $Q_{MH} = 5.10$ ;  $df = 1$ ;  $P = 0.02$ ). No significant date effect was observed for upwind flight ( $Q_{MH} = 0.09$ ;  $df = 1$ ;  $P = 0.77$ ). Again, because all moths exhibiting upwind flight also made source contact with the pheromone lures, no stratified analysis was possible for the source contact behavior.

## Discussion

We tested Rb dosage levels to determine a dose that would result in moths sufficiently labeled for positive identification in mark-release-recapture studies with no

Table 3. Linear regression parameter estimates of Rb persistence in adult *G. molesta* fed diet enriched with 6,000 mg RbCl/L liquid diet at three different times (24, 96, 128 h) following adult eclosion.

Sex	Parameter	Intercept	Parameter estimate			Overall model	
	estimate ( $\pm$ SE)	estimate ( $\pm$ SE) *	T-value	df	P-value	R <sup>2</sup>	RMSE
Female	-0.004 (0.001)	7.8 (0.10)	-4.10	1	< 0.0001	0.15	0.55
Male	-0.004 (0.001)	7.5 (0.14)	-3.89	1	0.0003	0.22	0.45

\* T-values for all intercept parameter estimates were significant at  $P = 0.05$  with 1 df.



Table 4. Effect of Rb labeling (6,000 mg/L liquid diet) on male *G. molesta* flight behavior in response to a pheromone lure in a wind tunnel. Percentages (and numbers) of moths exhibiting sequential behaviors are calculated based on the number of moths completing the previous behavior in the sequence (Baker and Cardé 1979).

Treatment	% exhibiting wing-fanning (initial no.) <sup>†</sup>	% exhibiting take-off	% exhibiting lock-on	% exhibiting upwind flight	% exhibiting source contact <sup>‡</sup>
0	95.5 (89) NS	74.1 (63) NS	23.8 (15)*	93.3 (14) NS	100 (14)
6,000	87.8 (90)	78.5 (62)	41.9 (26)	96.2 (25)	100 (25)

<sup>†</sup> "NS" indicates no significant differences in the number of moths in each Rb treatment based on the number completing the previous behavior; and "\*" indicates significance at  $P = 0.05$  according to G<sup>2</sup> statistics from log-linear analysis of 2 × 2 tables ( $P = 0.05$ ) (Stokes et al. 2000).

<sup>‡</sup> Because every moth exhibiting upwind flight also made source contact, no 2 × 2 table analysis was possible.

perceivable detriment to development or longevity. The higher numbers of moths labeled at 6,000 ppm suggest that this dosage would result in fewer samples being labeled below the threshold. At all three dosages of Rb in our study, the data indicated that females incorporated more Rb into their tissues than males (Table 1). This finding is consistent with results found by previous researchers (Fleischer et al. 1989, Knight et al. 1989, Polavarapu et al. 1992, and Qureshi et al. 2004) regardless of dietary media or methods of Rb analyses. Knight et al. (1989) suggested that female reproductive tissues may absorb Rb selectively although in female *Diatraea grandiosella* Dyar females having already oviposited still retained higher levels of Rb than males (Qureshi et al. 2004). Further experimentation would establish if this is the case for female *G. molesta*.

Although Rb did not significantly affect total larval-to-adult longevity, Rb negatively affected the adult longevity of both sexes of *G. molesta*, also observed among female *P. idaeusalis* at 6,000 ppm (Knight et al. 1989). However, the significant regression parameters are on the order of  $1 \times 10^{-6}$  and represent numerically low incremental changes (Table 2). The difference in mean adult longevity in the 0 and 6,000 ppm treatments appears as 2.1 and 2.9 d in females and males, respectively. None of the pupae were visibly stunted, deformed or discolored. Our results are inconsistent with those of Polavarapu et al. (1992), who found that 5,000 ppm RbCl did not affect adult longevity of either sex of *C. curvalana* although their use of foliage as a dietary medium may have affected Rb uptake. Berry et al. (1972) showed that diet treated with 7,000 ppm Rb resulted in 2x the amount of Rb found in labeled male *Trichoplusia ni* (Hübner) when compared with the same dosage applied in foliar sprays of cotton. If Rb is considered for labeling endogenous *G. molesta* by foliar or fruit application, a comparable study would determine whether similar inconsistencies exist.

Days to weeks may pass between the exposure of the immature insects to Rb and the subsequent capture of labeled moths (Van Steenwyk et al. 1978). Polavarapu et al. (1992) demonstrated that adult *C. curvalana* meeting labeling criteria could be captured up to 15 d from the onset of flight in labeled host crops. Steiner and Yetter (1933) found that in 7 releases of male and female *G. molesta* marked topically with liquid dye, the number of days to recapture of the last individual following release in peach orchards was  $8 \pm 3$  d. According to our results, male and female *G. molesta*

labeled at the level of 6,000 ppm RbCl in their diet would live long enough to be recaptured beyond 11 d.

The sequence of flight behaviors from first exposure to pheromone stimuli to contacting the pheromone source in *G. molesta* has been well-quantified (Baker and Cardé 1979). We are unaware of any literature describing the effects of Rb on male pheromone reception. A commercially-available synthetic pheromone blend was used in this study to mimic pheromone-baited traps used by pest managers in the field. However, the commercial blend contains a suboptimal amount of the important Z8 - 12:OH component which should be at ~10% of the pheromone blend for optimal attraction of male *G. molesta* (Linn and Roelofs 1983). This fact probably accounts for the relatively low responses of male moths in our wind tunnel study but would reflect what both labeled and unlabeled males would orient to in monitoring traps in the field (Evenden and McLaughlin 2005).

The higher incidence of lock-on and subsequent behaviors among Rb-labeled as compared with unlabeled moths is puzzling. However, Rb applied externally to invertebrate giant axon preparations was shown to extend action potentials (Baker et al. 1962). If such an effect also occurs in *G. molesta* an alteration in labeled moths' olfaction and resulting flight behavior may be a consequence. No significantly different treatment effects were found in behaviors preceding or following lock-on, suggesting that lock-on was the behavioral stage at which Rb labeling had an effect. Given the marginally significant difference more experimentation has merit. If subsequent experimentation should bear out significant behavioral effects, the implications of using pheromone traps as a recovery tool for Rb-labeled males would also require additional investigation.

Based on our results, male and female *G. molesta* may be labeled with 3,000 or 6,000 mg RbCl / L liquid diet as larvae without seriously adverse developmental or reproductive effects. Greater consistency was achieved at the higher dose and would be more advisable despite the doubling of Rb and consequently, the increased financial cost. Although Rb levels in adults decreased significantly after 24 h, all male and the majority of female moths retained Rb higher than their respective labeling standards through 168 h (7 d) following adult eclosion. Generally, Rb did not affect the males' ability to recognize and fly to an upwind source of pheromone, but labeled male moths appeared to be more likely to exhibit the lock-on and subsequent behaviors in the flight sequence. *Grapholita molesta* appears to be a good candidate for labeling with Rb for the purpose of mark-release-recapture studies. However, the nature of the heightened response of labeled males to sex pheromone lures should be further characterized to avoid misinterpreting trap-count data from such field trials.

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