

## THE USES OF ELEMENTAL MARKING FOR INSECT DISPERSAL AND MATING COMPETITIVENESS STUDIES: FROM THE LABORATORY TO THE FIELD

R. A. Van Steenwyk

Department of Entomological Sciences  
University of California  
Berkeley, California 94720

### ABSTRACT

Rubidium (Rb), an alkali metal with chemical properties similar to those of potassium (K), is uniquely suited as a marker element. Rb is rapidly absorbed and translocated by plants. Insects which feed on Rb-marked plants will themselves become marked. Since Rb is usually not naturally abundant in insects, Rb-marked insects can be distinguished from their unmarked counterparts. Rb has been successfully used to investigate intra and interfield dispersal and mating competitiveness of laboratory-reared and sterilized insects. Possible limiting factors in the use of Rb are high and variable background levels, rapid elimination of Rb once the marked insect leaves the Rb source, and detrimental biological or behavioral effects when administered in large amounts. Cesium (Cs), an alkali metal with chemical properties similar to those of Rb and K, has been proposed as a second internal marker. Cs is absorbed by plants when applied in an aqueous solution and translocated throughout plant tissue. However, Cs is more toxic than Rb and does not translocate within plants as well as Rb.

### INTRODUCTION

The use of rubidium (Rb) as an internal marker for studying insect dispersal was first proposed by Berry et al. (1972). Rb is an alkali metal with chemical properties similar to those of potassium (K). Rb is rapidly absorbed by plants when applied in an aqueous solution and translocated throughout the plant tissue (Levi 1970, Wallace 1968). Insects which feed on Rb-enriched plant tissue will become marked with Rb. Since the background Rb level in insects is usually quite low, Rb-marked insects can be distinguished from their unmarked counterparts. Other properties of Rb which make it ideally suited as a marker for large field studies of insect dispersal are that Rb is nonradioactive, has low mammalian toxicity, is not phytotoxic (Berry and Smith 1969), does not affect the behavior of the marked insect when administered in moderate amounts, and is relatively inexpensive (\$250/kg). These characteristics make Rb uniquely suited as a marker for dispersal studies and eliminate many of the problems associated with other marking techniques. Rb can also be used to study mating competitiveness of laboratory-reared and sterilized insects since significant amounts of Rb are transferred from Rb-marked males to unmarked females during mating (Van Steenwyk et al. 1979).

### BACKGROUND RUBIDIUM LEVELS

For the Rb marking technique to be successfully utilized in assessing insect dispersal, the Rb content of marked insects must be increased sufficiently above the background Rb content for the expected field life of the marked insects. The minimum Rb level to distinguish marked from unmarked individuals was defined by Stimmann (1974) as the mean background content plus 3 standard deviations of that mean. This minimum mark level would

result in an error rate of 0.13%, or 1 individual out of 770 individuals would be expected to exceed this level, assuming a normal distribution.

The background Rb concentration may vary widely among different insect species, host plants and geographical regions (Table 1). There appears to be considerable variation in mean background Rb concentration among different species in the same location. In Riverside, CA, Berry et al. (1972) reported 0.9  $\mu\text{g Rb/g}$  in *Trichoplusia ni* (Hübner) while Stimmann (1974) reported 9.2  $\mu\text{g Rb/g}$  in *Pieris rapae* (L.). Considerable variation in mean background Rb content also exists for the same species in different geographical regions of the United States. Berry et al. (1972) found the mean background Rb concentration for *T. ni* ranged from 0.8  $\mu\text{g Rb/g}$  in Hastings, FL to 3.9  $\mu\text{g Rb/g}$  in Waco, TX. These differences in Rb concentration may be due to selective absorption of Rb by the host plant. Collander (1941) reported that Rb is selectively absorbed by a wide variety of plant species and that Rb is absorbed at about the same rate as potassium. Newton (1928) reported that wheat and barley contained approximately 40% more potassium than corn or beans when grown in the same nutrient solution. Fleischer et al. (1986) reported 3.8  $\mu\text{g Rb/g}$  in *Lygus lineolaris* (Palisot de Beauvois) reared on cotton while 5.2  $\mu\text{g Rb/g}$  was reported when *L. lineolaris* was reared on mustard at the same location.

The mean background content of parasitic Hymenoptera appears to be high. Payne and Wood (1984) reported a background level of 107.3  $\mu\text{g Rb/g}$  for *Calliephialtes grapholithae* (Cresson), a larval parasitoid of *Cydia caryana* (Fitch). The high Rb concentration in *C. grapholithae* can be attributed to the high background Rb concentration of *C. caryana* (122.4  $\mu\text{g Rb/g}$ ). The high background Rb concentration in *C. caryana* can be attributed to the Rb concentration of its host, pecan shuck tissue. Payne and Wood (1984) reported that the background Rb concentration of pecan shuck tissues was 28.6  $\mu\text{g Rb/g}$  while that of leaves and kernels was 2.8 and 4.5  $\mu\text{g Rb/g}$ , respectively. Jackson et al. (1988) reported 75.8  $\mu\text{g Rb/g}$  for *Anaphes ovijentatus* (Crosby & Leonard), an egg parasitoid of *Lygus* spp. The cause of high background Rb concentration in *A. ovijentatus* cannot be determined since the background Rb concentration of *Lygus* spp. eggs or host plant tissue (alfalfa) was not reported.

A knowledge of the background Rb content is an essential requirement for successful utilization of the Rb marking technique. The amount of Rb needed to mark an insect for its expected field life must be increased if the insect has high background Rb content. If an increased amount of Rb needed to mark the insect causes deleterious effects to the biology or behavior of the marked insect, then the Rb marking technique should not be used.

#### RATE OF RUBIDIUM ELIMINATION

A second limiting factor of the Rb marking technique is the rate of Rb elimination from the marked insects. If Rb is eliminated from marked insects at a rate high enough to cause their Rb content to fall below the critical value (mean background content plus 3 standard deviations) during the field life of the insects, then information developed from field dispersal studies could be misleading.

Rb is eliminated from marked adult insects by excretion, oviposition and mating. The rate of elimination is dependent on feeding habits of the adult insects after leaving the Rb source and whether the insects acquired the Rb by developing on a Rb source as immatures or feeding on the Rb source as adults.

Insects which are reared on Rb-enriched sources and then fed for varying periods of time as adults on a food source without Rb retain sufficient amounts of Rb to distinguish them from unmarked individuals for varying periods of time. Graham and Wolfenbarger (1977) reported distinguishing amounts of Rb in adult *Heliothis virescens* (F.) for more than 7 days when larvae were reared on 10.0 or 1.0 g RbCl/l diet (ca. 21,000 or 2,100  $\mu\text{g Rb/g}$  dry weight basis). Van Steenwyk et al. (1978a) reported distinguishing amounts of Rb in adult *Pectinophora gossypiella* (Saunders) for more than 20 days when larvae were reared on  $1 \times 10^{-1}$ ,  $1 \times 10^{-2}$  and  $1 \times 10^{-3}$  M RbCl (ca. 8,547, 854.7 and 85.5  $\mu\text{g Rb/g}$  wet weight basis) diets. Burns et al. (1983) reported distinguishing amounts of Rb in adult *Ceratitis capitata* (Wiedemann) through 14 to 16 days when larvae were reared on 710  $\mu\text{g Rb/g}$  diet (wet weight basis).

The rate of Rb elimination appears to be rapid the first few days of adult life. The rate of elimination then slows for the remainder of the life of the insect. Van Steenwyk et al.

TABLE 1. Background Rubidium Concentration of Various Field-reared Adult Insect Species from Various Locations in the United States.

Species	Sex	Location	Host	Mean Rb ( $\mu\text{g/g}$ dry weight)	Ref. <sup>a</sup>
<i>Anaphes oviventatus</i>		Morana, AZ	<i>Lygus spp.</i> eggs	75.8	1
<i>Anthonomus grandis</i>		Brownsville, TX	Cotton	35	2
<i>Anthonomus grandis</i>		Brownsville, TX	Cotton	47	3
<i>Calliephialtes grapholithae</i>		Byron, GA	<i>C. caryana</i> larvae	107.3	4
<i>Cydia caryana</i>		Byron, GA	Pecan	122.4	4
<i>Epilachna varivestis</i>		Clemson, SC	Soybean	4.9 <sup>b</sup>	5
<i>Graminella nigrifrons</i>		Eatonton, GA	Johnsongrass	9.5	6
<i>Heliothis zea</i>		Brownsville, TX	Corn	31	7
<i>Leptinotarsa decemlineata</i>		Sunderland, MA	Potato	13.0	8
<i>Lygus lineolaris</i>		Belle Mina, AL	Cotton	3.8 <sup>c</sup>	9
<i>Lygus lineolaris</i>		Belle Mina, AL	Mustard	5.2	9
<i>Lymantria dispar</i>	M	Shenandoah Natl. Park, VA	Pin oak	0.8	10
<i>Lymantria dispar</i>	F	Shenandoah Natl. Park, VA	Pin oak	2.0	10
<i>Pectinophora gossypiella</i>	M	El Centro, CA	Cotton	0.5	11
<i>Pectinophora gossypiella</i>	F	El Centro, CA	Cotton	0.7	11
<i>Pectinophora gossypiella</i>	M	southern CA - western AZ	Cotton	0.2-1.1	11
<i>Pectinophora gossypiella</i>	F	southern CA - western AZ	Cotton	0.1-1.0	11
<i>Pieris rapae</i>		Riverside, CA	Mixed vegetables	9.2	12
<i>Spodoptera frugiperda</i>		Brownsville, TX	Corn	31	7
<i>Trichoplusia ni</i>		Hasting, FL	Unknown	0.8 <sup>d</sup>	13
<i>Trichoplusia ni</i>		Riverside, CA	Unknown	0.9	13
<i>Trichoplusia ni</i>		Overton, NV	Unknown	1.7	13
<i>Trichoplusia ni</i>		Charleston, SC	Unknown	2.0	13
<i>Trichoplusia ni</i>		Waco, TX	Unknown	3.9	13

<sup>a</sup> Numbers refer to Literature Cited: 1) Jackson et al. (1988), 2) Graham et al. (1978a), 3) Wolfenbarger et al. (1982), 4) Payne and Wood (1984), 5) Shepard and Waddill (1976), 6) Alverson et al. (1980a), 7) Graham et al. (1978b), 8) Voss and Ferro (1985), 9) Fleischer et al. (1986), 10) Fleischer, et al. (1989), 11) Van Steenwyk et al. (1978b), 12) Stimmann (1974), 13) Berry et al. (1972).

<sup>b</sup> Rb concentration in  $\mu\text{g/g}$  was calculated using 24.8 mg/adult beetle wet weight (Personal Communication, R. B. Hammond).

<sup>c</sup> Rb concentration in  $\mu\text{g/g}$  was calculated using 2.1 mg/adult bug (Fleischer et al. 1986)

<sup>d</sup> Rb concentration in  $\mu\text{g/g}$  was calculated using 39.5 mg/adult moth (Stimmann et al. 1973).

(1978a) found that adult *P. gossypiella*, which had developed as larvae on  $1 \times 10^{-2}$  M RbCl diet and fed a sucrose solution as adults, lost 42% of their Rb concentration by day 6 (as compared to day 0) while only an additional 22% was lost by day 20.

In the above studies, adults were fed solutions which contained no added K. When K is added to the adult food, Rb is more rapidly eliminated from the adult insects as compared to adults which were fed no added K. Fairbanks and Burch (1968) observed a more rapid rate of Rb elimination when K was increased in the adult food of *Drosophila melanogaster* (Meigen), *D. repleta* Wollaston and *Megaselia scalaris* (Loew). Also, Pearson et al. (1989) reported the Rb content of eggs laid by adult *Spodotera exigua* (Hübner), which developed as larvae on a 4.5 g RbCl/l (ca. 3,180.6  $\mu$ g Rb/g wet weight basis, or 10,622  $\mu$ g Rb/g dry weight basis) diet, was lower when adults were fed a sucrose solution containing 0.1% K than that of eggs from adults fed only sucrose solution. They reported 30% and 15% loss of Rb on day 2 (as compared to day 1) when adults were fed K-enriched sucrose or sucrose alone, respectively. By day 7, an additional 43% and 5% of the Rb was lost when adults were fed K-enriched sucrose and sucrose alone, respectively. Thus, Rb appears to be eliminated at a rapid rate in the first few days of adult life when insects are reared on Rb-enriched food as immatures and fed solutions with or without K as adults. The initial Rb elimination is probably from Rb contained in the gut and hemolymph. As the adult ages, the rate of Rb elimination slows. This Rb is probably contained in the insect's tissue and may have replaced K in the cell metabolism in the developing immature. The rate of Rb elimination in adults is accelerated with increasing levels of K in the adult food.

Insects which acquire Rb through adult feeding over a brief period may not have sufficient time to incorporate the Rb into body tissues. After the adult leaves the Rb source, Rb may be eliminated at a rate such that sufficient Rb may not be retained to mark the insect for its expected field life. Fleischer et al. (1986) fed adult *L. lineolaris* for 2 days on Rb-enriched mustard plants. These adults were then fed for varying time periods on untreated green beans. They found that Rb was reduced to background levels within 2 to 3 days. Shepard and Waddill (1976) fed adult *Epilachna varivestis* (Mulsant) for 4 days on Rb-enriched bean plants and then fed them for varying time periods on untreated beans. The adults lost 77% of their Rb by day 4 (as compared to day 0) and 89% by day 34. However, the adults retained sufficient Rb to distinguish them from background levels for the 34 days. Culin and Alverson (1986) fed adult *Heliothis zea* (Boddie) for 12 h on enriched artificial nectar containing very high concentrations of Rb (5,000, 10,000 and 20,000 ppm RbCl, or 3,534, 7,068 and 14,136  $\mu$ g Rb/g). The moths were then fed unmarked nectar for varying periods of time. The moths contained sufficient Rb to mark them for their expected adult life of 12 days; however, increased mortality was observed at the 10,000 and 20,000 ppm RbCl levels.

The Rb content in adult insects which have fed on a Rb source only as adults is directly dependent on the Rb concentration in the source and the time the adults fed on the Rb source. If the adults are expected to feed on the Rb source for a brief period of time, then the Rb concentration of the source must be increased. If the increased amount of Rb needed to mark the insect causes deleterious effects to the biology or behavior of the marked insect, then the Rb marking technique should not be used.

## BIOLOGICAL EFFECTS OF RUBIDIUM

The administration of Rb at moderate concentrations to the larval diet or adult food does not appear to adversely affect the biology or behavior of the marked insect. However, when high concentrations of Rb are administered, adverse effects have been reported. Stimmann et al. (1973) found no significant differences in adult fecundity, fertility, longevity and pheromone response in *T. ni* when larvae were reared on artificial diets containing 0, 70, 700 and 7,000  $\mu$ g Rb/g (dry weight basis). When *T. ni* were reared on artificial diets containing 0, 3,500, 7,000, 14,000, 28,000, 42,000, 56,000 and 70,000  $\mu$ g Rb/g (dry weight basis), larval developmental time increased with increasing concentrations. Pupal developmental time did not increase. Adult deformity increased when larvae were fed diets containing Rb in concentrations above 28,000  $\mu$ g Rb/g, with only 10% of the adults fed the 70,000  $\mu$ g Rb/g diet appearing normal. Van Steenwyk et al. (1978a) reported no significant difference in adult survival and larval development in *P. gossypiella* when larvae were reared on artificial diets containing 0,  $1 \times 10^{-3}$ ,  $1 \times 10^{-2}$ , and  $1 \times 10^{-1}$  M RbCl (ca. 0, 85.5, 854.7

and 8,547  $\mu\text{g Rb/g}$  (wet weight basis) but complete larval mortality when larvae were reared on 85,470  $\mu\text{g Rb/g}$  (wet weight basis). Graham and Wolfenbarger (1977) reported no significant differences in *H. virescens* larval mortality, development, pupal size, adult eclosion, longevity or fecundity when larvae were reared on artificial diets containing 0, 0.1, 1.0, and 10.0 g RbCl/l (ca. 0, 210, 2,100 and 21,000  $\mu\text{g Rb/g}$  dry weight basis). Legg and Chiang (1984) reported no significant difference in *Ostrinia nubilalis* (Hubner) adult deformity, mortality or fecundity when larvae were reared on artificial diets containing 0 to 10,000  $\mu\text{g RbCl/g}$  (ca. 0 to 7,068  $\mu\text{g Rb/g}$  dry weight basis). Knight et al. (1989) reported increased larval developmental time with increasing Rb concentration when *Platynota idaeusalis* (Walker) larvae were reared on artificial diets containing 0, 600, 3,000 and 6,000 mg Rb/l wet weight basis (0, 3,540, 17,700 and 35,400  $\mu\text{g Rb/g}$  dry weight basis). However, there was no effect on pupal developmental time. Adult female longevity, number of egg masses and number of eggs were significantly lower at 6,000 mg Rb/l. Culin and Alverson (1986) fed adult *H. zea* Rb-enriched artificial nectar (5,000, 10,000 and 20,000 ppm RbCl, or 3,534, 7,068 and 14,136  $\mu\text{g Rb/g}$ ) and reported increased adult mortality at the 10,000 and 20,000 ppm RbCl levels.

#### FIELD DISPERSAL USING RUBIDIUM

Stimmann (1974) first demonstrated that Rb could be used to mark insects in the field. He applied RbCl to a collard field three times during June, 1972, which marked *P. rapae* from June through August. However, he made no attempt to follow the dispersal of these butterflies. Wolfenbarger et al. (1982) first demonstrated the utility of the Rb-marking technique in the field to assess insect dispersal. They applied RbCl to cotton about every 3 wk from April through August in 1973 and from April through mid-July in 1974 to a portion of a cotton field in Brownsville, TX and successfully followed the dispersal of *A. grandis* from 1973 through 1975. Van Steenwyk et al. (1978b) successfully assessed intra and interfield dispersal of male *P. gossypiella* within a growing season, dispersal of overwintering male moths and interfield dispersal of female moths. They applied RbCl weekly from mid-July to mid-September, 1975 and from July through September, 1976 to a cotton field in southern California. Graham et al. (1978b) assessed intercrop dispersal of adult male *H. zea*. They applied RbCl once in May, 1975 to a corn field and then monitored Rb-marked adult male moths over the next 7 weeks in nearby cotton fields. They found a considerable number of marked moths in the cotton fields.

Following the initial studies which demonstrated the utility of Rb in assessing insect dispersal, Alverson et al. (1980b) and Fleischer et al. (1988) assessed the intrafield dispersal from a central nursery host for *Graminella nigrifrons* (Forbes), and *L. lineolaris*, respectively. Alverson et al. (1980b) assessed the intrafield dispersal from Johnsongrass, the nursery host, to two adjacent corn fields. The Johnsongrass was treated with RbCl in June, 1978 and *G. nigrifrons* was collected at varying times and distances from the treated nursery host. They found that marked leafhoppers decreased exponentially as a function of distance from the treated source and that the marked leafhoppers assumed a more uniform distribution across the corn fields with time.

Fleischer et al. (1988) assessed the intracrop dispersal of adult *L. lineolaris* from Rb-enriched *Erigeron annuus* (L.) Persoon, the central nursery host, to surrounding cotton and nearby *E. annuus* refuges in 1983 and from Rb-enriched mustard, the central nursery host, to surrounding cotton with no refuges in 1984. In 1983, the *E. annuus* nursery host was treated twice with RbCl in late June and early July. The nursery host was destroyed in mid-July and *L. lineolaris* adults were monitored in the surrounding cotton and refuges for 2 days. They found that there was a large increase in the number of marked adults found in the refuges 1 and 2 days later. In 1984, the mustard was treated twice in late June, and the nursery host was destroyed in early July. Adult Rb-marked *L. lineolaris* were monitored for 3 days after nursery host destruction at varying distances and directions from the treated area. They found an exponential decrease of marked bugs with increasing distance from the treated area, and the dispersal fit a diffusion model. Voss and Ferro (1990) sprayed RbCl 4 times from late June through mid-August, 1985 and 7 times from mid-June through mid-August, 1986 to potato at South Deerfield, MA. They monitored the dispersal of *L. decemlineata* from the treated field and found that Rb-marking allowed for the determination of the origin of the adults and local flight activity but was inefficient for monitoring migratory flight.

Knight et al. (1990) monitored dispersal by locating egg masses deposited by Rb-marked laboratory-reared and released *P. idaeusalis*. They made two releases on 11 to 19 July and 5 to 15 September and then collected egg masses at varying distances and directions from the release points. They found that dispersal was primarily local, following an inverse distance function, and was affected by ambient air temperatures.

### MATING COMPETITIVENESS USING RUBIDIUM

Graham and Wolfenbarger (1977) first demonstrated that elevated amounts of Rb were contained in spermatophores when male *H. virescens* were reared on 10.0 g RbCl/l enriched diet. However, they reported a mean of 64% (38-80%) of the spermatophores were marked with Rb and concluded that spermatophores were not marked consistently enough to be used in mating studies. Van Steenwyk et al. (1978a) reported consistent (100%) Rb-marked female abdomens of *P. gossypiella* for 16 days after mating when males were reared on  $1 \times 10^{-1}$  M RbCl-enriched diet and mated to untreated females. When males were reared on  $1 \times 10^{-2}$  M RbCl-enriched diet and mated to untreated females, the female abdomens were marked for at least 10 days. When males were reared on  $1 \times 10^{-3}$  M RbCl-enriched diet and mated to control females, the female abdomens were not marked. Also, Pearson et al. (1989) reported consistent (100%) Rb-marked *S. exigua* females which were mated to males reared on Rb-treated cotton and alfalfa plants. Thus, it appears that if sufficient amounts of Rb are incorporated into males, then sufficient amounts of Rb will be transferred in mating to mark unmarked females for their expected field life.

Van Steenwyk et al. (1979) assessed mating competitiveness of laboratory-reared and sterilized *P. gossypiella* which are used in a sterile moth release program in California. Male *P. gossypiella* were reared on artificial diet containing 60 g RbCl/kg diet and Calco<sup>tm</sup> Red dye. Females were reared similarly on diet with no added Rb. Both male and female moths were sterilized with 20 Krad of gamma irradiation. Various rates of laboratory-reared and sterilized males were released into field cages with a constant number of native males and females. The experiment was repeated with laboratory-reared and sterilized females. In a third experiment, various rates of laboratory-reared and sterilized males and females were released with a constant number of native males and females.

The results showed that laboratory-reared and sterilized males were less competitive in mating with native females than their native male counterparts, that laboratory-reared and sterilized females were more competitive in attracting males than their native female counterparts and that the competitiveness of the combination of both laboratory-reared and sterilized males and females was approximately equal to that of their native counterparts. They concluded that female *P. gossypiella* were the most important component in the sterilized moth release program.

### OTHER ELEMENTAL MARKERS

The use of cesium (Cs) as a second internal marker was first proposed by Moss and Van Steenwyk (1982), although Stimmann (personal communication) investigated the uses of Cs in the early 1970's. Cs is an alkali metal with chemical properties similar to those of Rb and K. Cs is absorbed by plants when applied in an aqueous solution and translocated throughout the plant tissue (Middletown 1958, Wallace 1968, Levi 1970). Insects which feed on Cs-enriched plant tissue will become marked with Cs. Since the background Cs level is usually quite low (Goldschmidt 1954), Cs-marked insects can be distinguished from their unmarked counterparts. Thus, Cs can be used in conjunction with Rb to produce three distinct marks (Cs alone, Rb alone and Cs/Rb combination).

Moss and Van Steenwyk (1982), using *P. gossypiella*, and Moss and Van Steenwyk (1984), using *T. ni*, reported that adults of both species which were reared on  $1 \times 10^{-2}$  and  $1 \times 10^{-3}$  M CsCl (ca. 1,330 and 133  $\mu$ g Cs/g wet weight basis) diet were sufficiently marked to last their expected field life. Adult *T. ni* which were reared on greenhouse-grown cotton plants treated with 1,000, 5,000 and 10,000  $\mu$ g Cs/ml and field-grown cotton plants treated with 1.25, 2.5 and 5.0 kg CsCl/ha were marked for their expected field life. However, adult *P. gossypiella* were marked for their expected field life when reared on greenhouse-grown cotton plants treated with only 5,000 and 10,000  $\mu$ g Cs/ml and field-grown cotton plants

treated with only 5.0 kg CsCl/ha. Hayes (1989) reported 100% marked *H. virescens* adults when larvae were reared on a diet containing 1,580 µg Cs/g (dry weight). However, eggs from these females and spermatophores from these males were not marked with Cs 100% of the time.

Cs appears not to be translocated throughout the plant to the same degree as Rb. Moss and Van Steenwyk (1982) reported 1.2 and 5.6 µg Cs/g in adult *P. gossypiella* when larvae were reared from field-grown cotton plants treated with 2.5 and 5.0 kg CsCl/ha, respectively, while Van Steenwyk et al. (1978a) reported 15.5 µg Rb/g in adult *P. gossypiella* when larvae were reared from field-grown cotton plants treated with 2.5 kg RbCl/ha. However, Moss and Van Steenwyk (1984) reported 102.4 and 328.2 µg Cs/g in adult *T. ni* when larvae were reared from greenhouse-grown cotton plants treated with 1,000 and 10,000 µg Cs/ml, respectively, while Stimmann et al. (1973) reported 97 and 596 µg Rb/g in adult *T. ni* when larvae were reared from greenhouse-grown plants treated with 1,000 and 10,000 µg Rb/ml. The higher Cs levels found in *T. ni*, which is an external foliage feeder, as compared to the Cs levels in *P. gossypiella*, which is an internal fruit feeder, can be attributed to lower translocation of Cs in the cotton plant as compared to Rb. Moss and Van Steenwyk (1982) reported significantly reduced Cs concentration in cotton seed and lint as compared to old or new foliage when cotton plants were treated with various rates of CsCl. Thus, the use of Cs as a second marker element may require that greater amounts of Cs be applied as compared to Rb, particularly in marking internal fruit feeding insects.

Cs appears to adversely affect the biology of insects at lower concentrations than Rb. Moss and Van Steenwyk (1982) found lower larval survival and increased development time in *P. gossypiella* when larvae were reared on a diet containing  $5 \times 10^{-2}$  M CsCl (ca. 6,650 µg Cs/g wet weight basis) while Van Steenwyk et al. (1978a) found no adverse biological effects in *P. gossypiella* when larvae were reared on a diet containing  $1 \times 10^{-1}$  M RbCl (ca. 8,500 µg Rb/g wet weight basis). Moss and Van Steenwyk (1984) found complete larval mortality in *T. ni* when larvae were reared on a diet containing  $1 \times 10^{-1}$  M CsCl (ca. 13,300 µg Cs/g wet weight basis) while Stimmann et al. (1973) found increased larval development time and adult deformity in *T. ni* when larvae were reared on 28,000 or greater µg Rb/g.

## CONCLUSION

Rb provides a powerful tool to assess insect dispersal and mating competitiveness of laboratory-reared and sterilized insects. Rb can be used as a replacement for P32 and provides similar types of information without the environmental and health concerns of P32. Since Rb can mark large numbers of insects at a relatively low cost without modifying the behavior of the marked insect, Rb can replace various dusts, dyes and tags, all of which may greatly modify the behavior. The problem of high and variable background levels and rapid Rb elimination from the marked insect may be compensated for by increasing the number of Rb applications or increasing the Rb concentration per application so that more Rb is incorporated into the marked insect. Cs is a possible second elemental marker which has the same advantages as Rb in assessing insect dispersal and mating competitiveness of laboratory-reared and sterilized insects. However, a great deal of additional research is needed before the utility of Cs can be established.

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