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In-Field Labeling of Western Corn Rootworm Adults (Coleoptera: Chrysomelidae) with Rubidium

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ABSTRACT Field and laboratory studies were conducted in 2000 and 2001 to determine the feasibility of mass marking western corn rootworm adults, *Diabrotica virgifera virgifera* LeConte, with RbCl in the field. Results showed that application of rubidium (Rb) in solution to both the soil (1 g Rb/plant) and whorl (1 g Rb/plant) of corn plants was optimal for labeling western corn rootworm adults during larval development. Development of larvae on Rb-enriched corn with this technique did not significantly influence adult dry weight or survival. Rb was also highly mobile in the plant. Application of Rb to both the soil and the whorl resulted in median Rb concentrations in the roots (5,860 ppm) that were 150-fold greater than concentrations in untreated roots (38 ppm) 5 wk after treatment. Additionally, at least 90% of the beetles that emerged during the first 3 wk were labeled above the baseline Rb concentration (5 ppm dry weight) determined from untreated beetles. Because emergence was 72% complete at this time, a significant proportion of the population had been labeled. Results from laboratory experiments showed that labeled beetles remained distinguishable from unlabeled beetles for up to 4 d postemergence. The ability to efficiently label large numbers of beetles under field conditions and for a defined period with virtually no disruption of the population provides an unparalleled opportunity to conduct mark-recapture experiments for quantifying the short-range, intrafield movement of adult corn rootworms.

KEY WORDS western corn rootworm, *Diabrotica virgifera*, rubidium, mark-recapture, *Zea mays*

DEVELOPING EFFECTIVE PEST management strategies for insects requires a detailed understanding of their life-history and population dynamics. A key component of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, population dynamics in field corn, *Zea mays* L., is adult dispersal. Western corn rootworm adults are capable of both intra- (Darnell et al. 2000) and interfield dispersal (Godfrey and Turpin 1983, Naranjo 1991). Beetle movement between cornfields is often related to changes in corn plant phenology that affects food quality within the field (Hill and Mayo 1974, Witkowski et al. 1975, Godfrey and Turpin 1983, Naranjo 1991). Beetles tethered to flight mill systems have been used in the laboratory to study the effects of age and reproductive development on flight behavior and to assess differences in flight characteristics between sexes (Coats et al. 1986, Naranjo 1990a). Unfortunately, little data have been collected that quantify the timing and magnitude of intrafield beetle movement relative to emergence sites. This informa-

tion has become increasingly important with the development of rootworm-targeted transgenic corn hybrids and the need to design refuges that promote random mating between resistant and susceptible insects and delay the evolution of rootworm resistance (International Life Sciences Institute 1999, U.S. Environmental Protection Agency 2002).

Insect dispersal is often studied by the marking, release, and recapture of insects that have either been collected from field populations or reared in laboratory colonies (Southwood 1978, Graham et al. 1978a). Critical to the success of such studies is formulating a marking technique that can be efficiently applied, causes minimal disruption to the population, persists for a known length of time, and does not cause any negative fitness effects (Akey 1991). Marking techniques previously developed for western corn rootworms in corn include the application of fluorescent dusts to field-collected beetles (Naranjo 1990b, Oloumi-Sadeghi and Levine 1990) and feeding beetles artificial diets treated with colored dyes (Naranjo 1990b, Lance and Elliott 1990). Although both techniques were shown to adequately mark the insects, they both have critical disadvantages that limit their use for mark-recapture studies. First, the number of

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insects that can be manually collected from the field or reared in the laboratory limits the number of insects that can be released. More importantly, movement of insects marked with these techniques is confounded by either the physical disruption of the population that occurs at the time of release or the unnatural, laboratory conditions in which the insects were reared.

In other cropping systems, labeling phytophagous insects with elemental markers, such as rubidium (Rb), has provided an economical, nondisruptive, and environmentally safe method for marking large numbers of insects through their consumption of Rb-treated host plants (Berry et al. 1972, Hayes 1991). Rb is a rare element found at relatively low but variable levels throughout the earth's surface (Stimmann 1991). In Illinois alone, Jones (1989) determined that the Rb concentration in the soil surface horizons ranged from 40 to 166 ppm across the state. As a result of this geographic variability, when Rb is used in mark-recapture studies, it is necessary to determine the baseline concentration of Rb present in the system above which an insect is considered positively labeled. The baseline Rb concentration is calculated as the mean concentration in unlabeled, endogenous insect populations +3 SD above that mean (Van Steenwyk 1991). Because Rb has chemical properties similar to potassium, it can be applied to the host plant, be taken up systemically, and be accumulated in the insect as it feeds to concentrations higher than levels normally present in the endogenous population (Berry et al. 1972, Hopper 1991).

Such a marking technique should be conducive to labeling western corn rootworms because the larvae are subterranean, relatively immobile, and typically complete their development on corn roots. The objectives of this study were to (1) develop a technique to efficiently label western corn rootworm adults with Rb in the field; (2) quantify Rb uptake in corn and its partitioning to various plant structures; and (3) determine if the labeling technique would be feasible for studying intrafield movement of western corn rootworm adults.

Materials and Methods

Field studies were conducted in 2000 and 2001 at the University of Nebraska Agricultural Research and Development Center, near Mead, NE. In both years, plots were established in areas where soybean, *Glycine max* L., had been grown the previous season. The experimental design in both years was a randomized complete block with four treatments replicated four times. In 2000, treatments consisted of an untreated control and three rates of Rb (0.08, 0.40, and 0.80 g Rb/30.5-cm row) applied to the soil at the base of each plant. Plots to which treatments were applied contained three rows of corn each 6 m in length. In 2001, treatments were an untreated control and three Rb application methods: (1) 1 g Rb applied to the soil at the base of each plant; (2) 1 g Rb directed into the whorl of each plant; (3) 1 g Rb applied to the soil and 1 g Rb directed into the whorl of each plant. Plots to

which treatments were applied contained three rows of corn each 21 m in length. Soil type was a Sharpsburg silty clay loam. In April of both years, nitrogen was applied at a rate of 68 kg/ha, and the plot areas were disked and harrowed before planting. The corn hybrid 33G26 (Pioneer Hi-Bred International, Des Moines, IA) was planted in early May of both years using a four-row Kinze model 2100 (Kinze Manufacturing, Williamsburg, IA) planter with rows spaced 76 cm apart at a rate of 66,700 seeds/ha. A standard weed control program consisting of both a pre- and post-emergence herbicide application was used in both years. In 2000 and 2001, the plots received 26.4 and 23.9 cm of precipitation, respectively, during June and July as both rainfall and irrigation.

Field Study 2000. Western corn rootworm eggs were obtained from beetles collected near Concord, NE, in 1999 (French Agricultural Research, Lambert, MN). Eggs were suspended in a 0.125% solution of agar-water and manually injected with a large syringe \approx 10–15 cm into the soil at the base of each plant. All plants in the center row of each treatment were infested at a rate of 350 eggs per plant. Plants were at the two-leaf stage of growth at the time of infestation.

The Rb treatments were applied on 13 June to the infested corn plants at the six-leaf stage of growth and corresponded to the initiation of egg hatch. Technical grade RbCl (99% purity; Aldrich, Milwaukee, WI) was applied as a powder with a bicycle-applicator through a Noble metering unit (Noble Manufacturing, Sioux City, IA). The applicator had a single drop-tube that directed the dry RbCl to the soil at the base of each infested plant on one side of the row. Immediately after application, the plot was tilled with a row-crop cultivator to incorporate the Rb. A total of 1.1 cm of rainfall was received during the 12-h period after application.

The endogenous concentration of Rb in the soil was determined 4 d before the treatments were applied by removing a single soil core, 2.5 cm in diameter, from each treatment. Each core was taken 12 cm away from the base of a treated plant and was divided by depth into three subsamples, 0–20, 20–60, and 60–122 cm, respectively. To quantify changes in the Rb concentration of soil over time, a single core per treatment was also extracted 2 and 21 wk after treatment. However, for soil samples taken 2 wk after treatment, only two depths were sampled, 0–20 and 20–60 cm. Soil samples were dried at 65°C for 4–5 d and stored in sealed containers.

Partitioning of Rb in the plants was examined by removing tissue samples from two plants per treatment. Plants were sampled 4 d pretreatment, and at two and 8 wk posttreatment. Samples were removed from three positions: 1) roots; 2) lower leaf (lowest green leaf in its entirety); and 3) stalk (included node where lower leaf was attached plus internode below that point). Root samples were obtained by extracting each root mass from the soil and washing it free of soil with pressurized water. Each root sample consisted of a vertical cross-section of the nodal roots that were cut from one side of each root mass. Plant samples were

dried at 65°C for 4–5 d and stored in sealed containers.

Rb concentrations in newly emerged beetles were determined from beetles collected in two screen emergence cages per treatment, each with an area of 76 x 43 cm. Each cage was positioned across the row and was centered over a single plant, but encompassed an area equivalent to two plants (the center plant and half the root system of the plant located on each side). Cage placement required cutting the corn stalk near its base, setting the cage over the cut plant, and sealing the wood base with soil. Beetles were captured in a 473 ml glass jar attached to the screen at the top of each cage. Beetles were collected from the jars at 3–4-d intervals for 5 wk and stored at –20°C.

Field Study 2001. On 23 May, western corn rootworm eggs (same source as 2000) were manually infested to 20 consecutive plants in the center row of each treatment at a rate of 200 eggs per plant. Plants were at the two-leaf stage of growth at the time of infestation. On 7 June, the Rb treatments were applied to the infested plants at the six-leaf stage of growth. Technical grade RbCl was applied in solution to the infested plants with a Labmax 10-ml bottle top dispenser (VWR Scientific Products, Willard, OH). For treatments applied to the soil, the RbCl solution (10 ml per plant) was dispensed to the soil surface around the base of each infested plant. For the whorl treatments, 5 ml of the RbCl solution was dispensed directly into the whorl of each infested plant. The plot was tilled with a row-crop cultivator 5 d after treatment to incorporate the Rb applied to soil.

Rb concentrations in the soil were determined by extracting three soil cores per treatment 5 and 8 wk after treatment. Each core was taken 12 cm away from the base of a treated plant and was divided by depth into two subsamples, 0–20 and 20–60 cm, respectively. Soil samples were dried at 65°C for 4–5 d and stored in sealed containers. Three plants per treatment were sampled 5 and 8 wk after treatment to examine the partitioning of Rb in the plants. On each date, samples were removed from five positions per plant: (1) the roots; (2) lower leaf (the lowest green leaf in its entirety); (3) lower stalk (included node where lower leaf was attached plus internode below that point); (4) upper leaf (third leaf from top of the plant in its entirety); and (5) upper stalk (included node where upper leaf was attached plus internode below that point). Root samples were obtained using methods described in the 2000 study. Plant tissue samples were dried at 65°C for 4–5 d and stored in sealed containers. Rb concentrations in newly emerged beetles were determined from beetles collected in three screen emergence cages per treatment using methods described in the 2000 study. Beetles were collected from the cages at 2- to 3-d intervals for 6 wk and stored at –20°C.

Rb Extraction. Rb concentrations in the soil were determined using methodology similar to that described by Knudsen et al. (1982) for extracting potassium from soil. A 2-g subsample of dried soil was removed from each sample, ground to a powder, and

placed in a 50-ml polypropylene centrifuge tube with 20 ml of 1 N ammonium acetate (pH 7.0). The sample was vortexed at a high speed for 30 s and centrifuged at 4,000 rpm for 3 min. Immediately after centrifugation, 10 ml of supernatant was extracted from each sample and transferred to a new tube for analysis.

All dried plant samples were ground to a powder with an electric coffee bean grinder, and a 5-mg subsample was removed from each. Each subsample was placed in a 1.5-ml polypropylene centrifuge tube with 50 μ l of 70% nitric acid. After 12 h of digestion at room temperature, each tube was placed in a dry bath for 1 h at 80°C, after which 10 μ l of the digest was transferred to a new tube containing 340 μ l of double-distilled water for analysis.

The beetles were prepared by drying individuals for 24 h at 80°C. After drying, individual beetles were weighed and placed in a 1.5-ml polypropylene centrifuge tube with 75 μ l of 70% nitric acid. After 12 h of digestion at room temperature, the tubes were placed in a dry bath for 1 h at 80°C, after which 10 μ l of the digest was transferred to a new tube containing 340 μ l of double distilled water, which was used for analysis.

Rb concentrations in the soil, plant, and beetle samples were measured in a graphite furnace by atomic absorption (AA) spectroscopy using a Varian FS220 atomic absorption spectrometer (Varian, Palo Alto, CA). An auto-sampler automatically dispensed a volume of 15 μ l of each sample into the pyrolytic-coated, partitioned, graphite tube, where the sample was heated to 2,000°C through a series of nine temperature steps. Rb content was measured by the amount of energy absorbed at 780.0 nm. The standard solution was prepared by dissolving RbCl salt in 1% nitric acid. The analytical range for the AA was 10 ng/ml. Samples with Rb concentrations above this range were manually diluted and reanalyzed. Rb concentrations were reported in ppm dry weight for each sample.

Analysis. Rb concentrations in the soil, plants, and beetles were initially tested for normality by year, sampling date, and treatment using the Shapiro-Wilk W-statistic ($P \leq 0.05$) calculated with the univariate procedure in PC-SAS (SAS Institute 1999). Results indicated that only data from the untreated plots were normally distributed and that Rb concentrations within treatments were highly right-skewed (i.e., some samples with extremely high Rb concentrations). Standard transformation techniques were unsuccessful, so all data were analyzed using nonparametric randomized complete block analysis of variance (ANOVA) and the RANK procedure in PC-SAS ($P \leq 0.05$) (SAS Institute 1999). Nonparametric ANOVA is an alternative when the assumption of normality is not met and uses the rank of Rb concentrations among treatments, rather than their raw values, to calculate statistics (Conover and Iman 1981). Median Rb concentrations among treatments were compared using LSDs (LSD test; $P \leq 0.05$), and 95% CL calculated for treatment medians.

Rb Dynamics in Beetles. Three additional laboratory experiments were conducted to examine the Rb elimination and uptake rates in adult western corn

rootworms. Sixteen translucent plastic 5.8-liter boxes were each filled with 2,000 ml of dry, silty clay loam soil. Twelve boxes were each treated with 250 ml of a 10,000 ppm RbCl solution (99% purity; Aldrich, Milwaukee, WI). The remaining four boxes were untreated controls to which 250 ml of distilled water were added. Contents of each box were thoroughly mixed until the soil was uniformly moistened. After mixing, 400 ml of pregerminated corn seeds were evenly distributed on the soil surface of each box. The boxes were covered with lids and held at 21°C and a photoperiod of 16:8 (L:D) h. Beginning 6 d later, neonate western corn rootworm larvae were infested over a 6-d period until a total of 125 larvae had been added per box. Larvae were obtained from a nondiapausing colony maintained at the USDA Agricultural Research Service, Northern Grains Insects Research Laboratory, in Brookings, SD. After infestation, the soil was kept moist by misting the surface of each box with distilled water as needed. Seedling leaves were trimmed at 2- to 3-d intervals during the period of larval development. Once pupae were detected, all vegetation was cut at the soil surface and removed from the boxes. Throughout the emergence period, beetles were collected daily from each box, separated by sex, and maintained in 28-cm³ clear Plexiglas cages on a diet of fresh sweet corn ears and lettuce. Cages were held at 21°C and a photoperiod of 16:8 (L:D) h. After 0, 0.5, 1, 2, 3, 4, and 5 d of feeding on the sweet corn and lettuce, 25 males and 25 females per time interval were removed from the cages and stored at -20°C, and their Rb concentration determined using methods described in the previous section. Endogenous concentrations of Rb in the beetles were determined from 25 male and 25 female beetles collected from the untreated larval rearing boxes.

A second experiment was conducted to determine how feeding on Rb-enriched corn foliage as adults affected Rb concentrations in beetles previously labeled with Rb as larvae. Approximately 100 ml of pregerminated corn seeds were placed in the bottom of 300-ml plastic pots without drain holes. The seeds were covered with 200 ml of dry, silty clay loam soil. The soil was moistened by adding 50 ml of a 10,000 ppm RbCl solution to each pot. Pots were held for 7–10 d at 22°C and a photoperiod of 12:12 (L:D) h. Soil in the pots was kept moist by adding distilled water as needed. A total of 80 male and 80 female beetles were collected from the 12 larval rearing boxes treated with Rb. Males and females were placed in separate 28-cm³ cages that each contained a pot of the Rb-enriched corn seedlings. After 0, 0.5, 1, 2, and 3 d of feeding on the Rb-enriched seedlings, 16 beetles per time interval were removed from each cage and stored at -20°C, and their Rb concentration was determined.

A third experiment was conducted to examine the uptake of Rb in previously unlabeled beetles through their feeding on Rb-enriched corn foliage. A total of 60 male and 60 female beetles were collected from the four larval rearing boxes not treated with Rb. Males and females were placed in separate 28-cm³ cages that each contained a single pot of the Rb-enriched corn

Table 1. Median (95% CL) concentrations of Rb (ppm dry weight) in soil at three depths, 2 and 21 wk after treatment

Treatment (g Rb/30.5 cm row)	2 wk after treatment		
	0–20 cm	20–60 cm	60–122 cm
4 d pretreatment	6 (5–7)a	13 (9–14)a	10 (8–14)
Untreated	8 (7–38)ab	10 (9–11)a	—
0.08	94 (10–215)c	47 (10–108)a	—
0.40	23 (6–443)bc	13 (8–74)a	—
0.80	131 (19–188)c	31 (11–59)a	—
	21 wk after treatment		
4 d pretreatment	6 (5–7)a	13 (9–14)a	10 (8–14)a
Untreated	4 (3–5)a	10 (4–12)a	7 (6–8)a
0.08	5 (4–36)a	11 (6–12)a	9 (6–13)a
0.40	9 (4–53)ab	13 (8–15)a	11 (7–13)a
0.80	66 (8–200)b	10 (6–11)a	9 (7–12)a

Powdered RbCl was applied to the soil at three rates on 13 June 2000, Mead, NE ($n = 4$).

For each sampling date, medians within columns followed by the same letter are not significantly different (LSD test; $P > 0.05$).

seedlings. After 0, 0.5, 1, 2, and 3 d of feeding, 8–12 beetles per time interval were removed from the cages and stored at -20°C, and their Rb concentration was determined. Rb elimination and uptake rates in beetles were described with nonlinear regression models developed with SigmaPlot (SPSS Inc. 1999).

Results

Field Study 2000. Rb application rate significantly affected Rb concentrations in the soil measured 2 wk after treatment ($F = 7.76$; $df = 4, 27$; $P < 0.01$). However, neither depth nor the rate \times depth interaction was significant ($P > 0.05$). While all three rates significantly elevated median Rb concentrations in the upper 20 cm of soil compared with the level 4 d before treatment, the largest increase (20-fold) was observed for the highest rate of Rb applied (Table 1). Below 20 cm, there were no significant differences in the median Rb concentration of soil between treatments for samples extracted 2 wk after treatment (Table 1).

Analysis of soil extracted 21 wk after treatment indicated both a significant rate ($F = 4.20$; $df = 4, 42$; $P < 0.01$) and depth ($F = 3.74$; $df = 2, 42$; $P = 0.03$) effect on Rb concentrations of soil. However, the rate \times depth interaction was not significant ($P > 0.05$). On this date, comparison of treatment medians showed that Rb concentrations in the upper 20 cm had declined to levels equivalent to that measured 4 d before treatment for all but the highest Rb rate (Table 1). At this rate (0.80 g Rb/30.5 cm row), the median Rb concentration in the upper 20 cm of soil remained 10-fold above the concentration measured 4 d before treatment (Table 1). At depths below 20 cm, however, median Rb concentrations for all rates were equivalent to the level observed 4 d before treatment (Table 1).

The naturally occurring concentration of Rb in the plants 4 d before treatment was uniformly distributed across the three plant parts sampled, with median concentrations in the roots, stalks, and leaves equivalent to 6 ppm (Table 2). Analysis of plant samples

Table 2. Median (95% CL) concentrations of Rb (ppm dry weight) in roots, stalks, and leaves of corn plants 2 and 8 wk after treatment

Treatment (g Rb/30.5 cm row)	2 wk after treatment (10 leaf-stage)		
	Root	Stalk	Leaf
4 d pretreatment	6 (5-8)a	6 (5-8)a	6 (5-7)a
Untreated	9 (6-16)b	24 (22-29)b	9 (4-14)b
0.08	22 (9-42)c	23 (13-55)b	12 (8-56)b
0.40	17 (11-37)c	37 (17-55)b	9 (7-35)b
0.80	17 (11-39)c	41 (16-80)b	9 (7-43)b
8 wk after treatment (post-pollination)			
4 d pretreatment	6 (5-8)a	6 (5-8)a	6 (5-7)a
Untreated	6 (5-8)a	5 (4-14)a	4 (3-7)a
0.08	29 (7-107)b	8 (5-18)a	7 (5-13)a
0.40	14 (11-246)b	8 (5-29)a	6 (4-51)a
0.80	121 (66-1422)c	25 (10-133)b	19 (7-76)b

Powdered RbCl was applied to the soil at three rates on 13 June 2000, Mead, NE ($n = 16$).

For each sampling date, medians within columns followed by the same letter are not significantly different (LSD test; $P > 0.05$).

taken 2 wk after treatment indicated both a significant rate ($F = 27.69$; $df = 4, 102$; $P < 0.01$) and plant part ($F = 19.82$; $df = 2, 102$; $P < 0.01$) effect on Rb concentrations in the plants. However, the rate \times plant part interaction was not significant on either sampling date ($P > 0.05$). Two weeks after treatment, all three Rb application rates significantly increased median Rb concentrations in the roots above the concentration in the untreated control (Table 2). There were no significant differences, however, between treatments for median Rb concentrations in the stalks or leaves on this date (Table 2).

Analysis of plant samples taken 8 wk after treatment also revealed significant rate ($F = 27.57$; $df = 4, 102$; $P < 0.01$) and plant part ($F = 14.73$; $df = 2, 102$; $P < 0.01$) effects on Rb concentrations in the plants. On this date, all three application rates significantly elevated median Rb concentrations in the roots, with the highest rate increasing the median concentration 20-fold over the concentration in the untreated roots (Table 2). In the stalks and leaves, however, only the highest rate maintained median Rb concentrations that were significantly greater than concentrations

measured 4 d before treatment (Table 2). These results suggest that Rb applied to the soil as a powder may not be readily available for uptake by the plants, because the maximum concentration in the roots was not observed until 8 wk after treatment.

None of the Rb application rates significantly reduced western corn rootworm survival from egg to adult compared with survival on untreated plants (Table 3). Across treatments, survival rates ranged from 2 to 4%, which were very comparable to levels reported by Elliott et al. (1989) for western corn rootworm eggs infested in the field with a similar technique. The Rb concentration in newly emerged beetles from untreated plants averaged 2 ± 3 ppm dry weight. All three Rb application rates produced beetles with median Rb concentrations significantly greater than the median concentration in untreated beetles (Table 3). However, the percentage of newly emerged beetles that were labeled above the baseline concentration of 11 ppm (untreated mean + 3 SD) was relatively low. Percentages that were positively labeled from the low, medium, and high Rb application rates were only 18, 23, and 27%, respectively. The low labeling efficiency in beetles could relate to the delayed Rb uptake observed in the plants. Maximum Rb concentrations in the roots were not observed until 8 wk after treatment, which was after most of the larvae had completed development. Additionally, there were no significant differences in the average dry weight of beetles between treatments (Table 3). This suggests that at least one beetle fitness parameter, beetle size, was not negatively impacted by exposure to Rb-treated corn as larvae.

Field Study 2001. In 2001, the naturally occurring median concentration of Rb in the soil (8 ppm) was comparable to the endogenous median concentration observed in 2000 (10 ppm). However, application of Rb in solution greatly enhanced concentrations in the soil compared with the dry formulation applied in 2000. Analysis of soil extracted 5 wk after treatment indicated a significant treatment \times depth interaction ($F = 3.05$; $df = 3, 85$; $P = 0.03$). On this date, application of Rb in solution to the soil, whorl, or to both locations (soil + whorl) each resulted in median Rb

Table 3. Mean (\pm SE) dry weights, median (95% CL) Rb concentrations, total number of beetles emerged, and survival rate from egg to adulthood for western corn rootworm adults reared on corn labeled with three rates of RbCl in 2000 and three application methods in 2001, Mead, NE

Year	Treatment ^a	<i>n</i>	Beetle dry weight (mg)	Median beetle [Rb] (ppm dry weight)	Total emerged	Survival (%) ^b
2000	Untreated	66	2.2 \pm 0.13a	2 (1-2)a	123	2.2 \pm 0.52a
	0.08 g Rb	65	2.1 \pm 0.12a	5 (3-7)bc	139	2.5 \pm 0.84a
	0.40 g Rb	82	2.1 \pm 0.10a	4 (3-6)b	130	2.3 \pm 0.39a
	0.80 g Rb	102	2.2 \pm 0.09a	6 (5-8)c	203	3.6 \pm 0.89a
2001	Untreated	105	2.4 \pm 0.10a	1 (1-2)a	274	5.7 \pm 0.99a
	Soil	56	2.5 \pm 0.13a	12 (8-18)b	177	3.7 \pm 0.52ab
	Whorl	57	2.7 \pm 0.15a	41 (10-76)bc	106	2.2 \pm 0.80b
	Soil + whorl	85	2.7 \pm 0.13a	142 (48-229)c	188	3.8 \pm 1.23ab

For each year, values within a column followed by the same letter are not significantly different (LSD test; $P > 0.05$).

^a In 2000, RbCl applied as powder to soil at base of each plant (g Rb/30.5 cm row). In 2001, RbCl was applied in solution to either the soil (1 g Rb/plant), directed into the whorl (1 g Rb/plant), or to both locations (2 g Rb/plant).

^b In 2000 and 2001, western corn rootworm eggs artificially infested at 350 and 200 eggs per plant, respectively.

Table 4. Median (95% CL) concentrations of Rb (ppm dry weight) in soil at two depths 5 and 8 wk after treatment

Treatment	5 wk after treatment	
	0–20 cm	20–60 cm
Untreated	8 (6–10)a	8 (7–10)a
Soil (1 g Rb/plant)	35 (13–199)b	24 (18–37)b
Whorl (1 g Rb/plant)	34 (15–98)b	10 (9–19)c
Soil + whorl (2 g Rb/plant)	296 (20–639)b	54 (18–127)b
8 wk after treatment		
Untreated	9 (7–10)a	10 (9–11)a
Soil (1 g Rb/plant)	68 (23–304)bc	18 (15–52)b
Whorl (1 g Rb/plant)	61 (28–273)b	22 (13–40)b
Soil + whorl (2 g Rb/plant)	317 (80–771)c	22 (17–56)b

RbCl was applied in solution on 7 June 2001 either to the soil, directed into the whorl, or to both locations, Mead, NE ($n = 12$).

For each sampling date, medians within columns followed by the same letter are not significantly different (LSD test; $P > 0.05$).

concentrations at both depths that were significantly elevated above concentrations in the untreated plots (Table 4). The significant treatment \times depth interaction was attributed to median Rb concentrations for the untreated control and soil treatment remaining constant across depths, while median concentrations declined with depth for the whorl and soil + whorl treatments (Table 4). Although the rate of Rb applied was doubled for the soil + whorl treatment, the median Rb concentration in the upper 20 cm of soil was not significantly different from each application method alone (Table 4). Interestingly, these data also suggest that when Rb is applied only to the whorl, it can be translocated to the roots and exuded into the soil.

Analysis of soil extracted 8 wk after treatment also showed a significant treatment \times depth interaction ($F = 5.69$; $df = 3, 85$; $P < 0.01$). The interaction was explained by differential rates of decline in Rb content at increasing depth among treatments (Table 4). Additionally, all three Rb treatments did significantly elevate median Rb concentrations of soil at both depths above the concentration of the untreated control (Table 4), with the highest concentration present in the upper 20 cm for the soil + whorl treatment. These data indicate that elevated Rb concentrations in

the upper 20 cm of soil can be maintained throughout the period of peak larval development by applying Rb in solution to both the soil and whorl of corn plants.

Application of Rb in solution also appeared to enhance Rb uptake by the corn plants. Analysis of plants 5 wk after treatment indicated that all three treatments significantly increased median Rb concentrations in all plant parts above concentrations in the untreated control (Table 5). For all treatments, median Rb concentrations were also numerically higher in the roots compared with the above-ground plant parts (Table 5). Roots from the soil + whorl treatment contained a median Rb concentration that was 150-fold greater than the concentration in untreated roots (Table 5). There was also a significant treatment \times plant part interaction on Rb concentration ($F = 2.74$; $df = 12, 217$; $P < 0.01$). For the untreated control and soil treatment, median Rb concentrations declined from the roots to the lower stalk and remained at relatively low and consistent levels across all the above-ground plant parts (Table 5). For the whorl and soil + whorl treatments, however, median Rb concentrations declined from the roots to the lower stalk, but remained relatively high across the remaining three above-ground plant parts (Table 5). These data seem to indicate greater translocation of Rb downward from the foliage to the roots compared with upward movement from the roots to the foliage.

Although Rb content had declined by 8 wk after treatment, all three treatments maintained Rb concentrations in all plant parts that were significantly elevated above concentrations in untreated plants (Table 5). Except for the lower leaf, application of Rb to both the soil and whorl of plants provided superior Rb concentrations in all plant parts compared with the application of Rb to each location alone (Table 5). These data also suggest that elevated Rb concentrations in the roots can be maintained throughout the period of peak larval development by applying Rb in solution to both the soil and whorl of corn plants.

Application of Rb in solution dramatically improved detectable Rb concentrations in adult western corn rootworms compared with the dry formulation of RbCl applied in 2000 (Table 3). The highest median

Table 5. Median (95% CL) concentrations of Rb (ppm dry weight) in the root, lower stalk, lower leaf, upper stalk, and upper leaf of corn plants 5 and 8 wk after treatment

Treatment	5 wk after treatment (13 July)				
	Root	Lower stalk	Lower leaf	Upper stalk	Upper leaf
Untreated	38 (20–55)a	12 (10–16)a	12 (8–18)a	21 (17–38)a	11 (7–18)a
Soil	1426 (486–2037)b	178 (135–258)b	150 (59–230)b	340 (219–556)b	213 (109–409)b
Whorl	2295 (1694–6844)c	736 (537–1882)c	2017 (1643–3598)c	868 (557–3932)c	730 (457–2247)c
Soil + whorl	5860 (3561–7078)c	932 (677–2234)c	2052 (1069–3375)c	2334 (1493–4229)d	1514 (1220–2831)d
8 wk after treatment (1 August)					
Untreated	11 (5–18)a	4 (3–6)a	6 (5–8)a	6 (4–10)a	7 (4–8)a
Soil	1303 (633–2052)b	75 (41–106)b	71 (43–98)b	123 (52–161)b	109 (62–188)b
Whorl	1840 (835–3636)c	369 (121–536)c	1806 (473–3993)c	368 (99–757)c	545 (196–668)c
Soil + whorl	2774 (2582–6411)c	649 (215–734)d	1268 (483–2435)c	552 (231–1182)d	788 (405–2001)d

RbCl was applied in solution on 7 June 2001 either to the soil (1 g Rb/plant), directed into the whorl (1 g Rb/plant), or to both locations (2 g Rb/plant), Mead, NE ($n = 12$).

For each sampling date, medians within columns followed by the same letter are not significantly different (LSD test; $P > 0.05$).

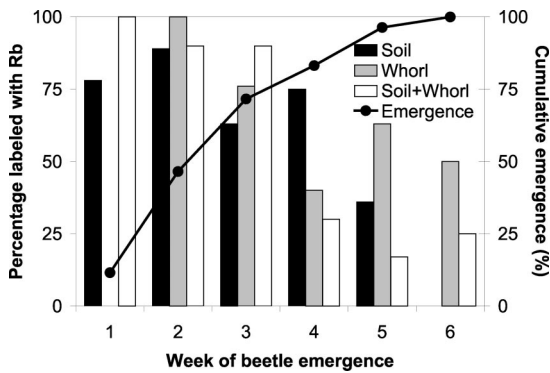


Fig. 1. Weekly (11 July–17 August) percentage of newly emerged western corn rootworm adults with Rb concentrations above the baseline concentration of untreated beetles (5 ppm dry weight). RbCl was applied in solution to either the soil (1 g Rb/plant), directed into the whorl (1 g Rb/plant), or to both locations (2 g Rb/plant). Mead, NE, 2001.

Rb concentration in beetles was obtained with the soil + whorl treatment. However, this Rb concentration was not statistically different from the concentration achieved with the whorl treatment alone (Table 3). There were no significant differences in the dry weights of beetles between treatments, and survival from egg to adult was generally unaffected by the treatments (Table 3). However, the whorl treatment alone did significantly reduce adult emergence relative to the untreated control (Table 3).

In 2001, initial beetle emergence occurred on 11 July, nearly 5 wk after the Rb treatments were applied, and continued for 6 wk. The mean \pm SD Rb concentration of the untreated beetles was 2 ± 1 ppm dry weight. The baseline Rb concentration above which a beetle was considered labeled was 5 ppm, equivalent to 3 SD above the mean of the untreated beetles. All three treatments produced beetles that were positively labeled throughout the 6-wk emergence period (Fig. 1). However, when Rb was applied both to the soil and the whorl, 90% or more of the beetles that emerged during the first 3 wk contained Rb concentrations above the baseline concentration (Fig. 1). This represents a significant proportion of the population that was labeled, considering emergence was 72% complete by this time (Fig. 1).

Rb Dynamics in Beetles. Rb concentrations in newly emerged beetles reared on roots of Rb-treated corn seedlings as larvae and transferred to a clean diet declined exponentially (Fig. 2). Rb concentrations were not significantly different between males and females ($F = 0.59$; $df = 1, 132$; $P > 0.05$), so a single elimination curve was fitted to data across sexes (Fig. 2). The mean \pm SD Rb concentration of untreated beetles was 1.4 ± 1 ppm dry weight. Based on these values, the baseline Rb concentration above which a beetle was considered labeled was 4 ppm (mean + 3 SD). This was very comparable to the baseline concentration of 5 ppm obtained in the 2001 field study. If a baseline concentration of 5 ppm were assumed, labeled beetles would remain distinguish-

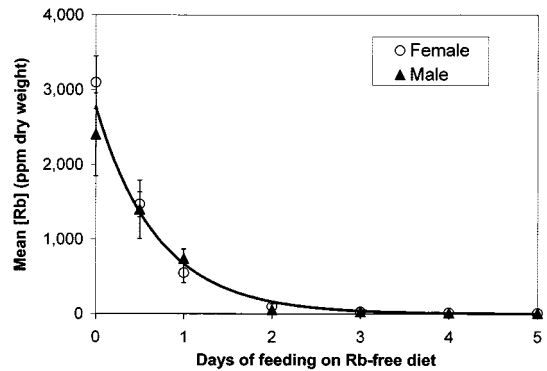


Fig. 2. Rb elimination rate in western corn rootworm adults after being reared on Rb-enriched corn seedlings as larvae and transferred to a clean diet of sweet corn and lettuce at emergence. Elimination followed an exponential decay model: $y = 2,722 e^{(-1.42 * x)}$; ($R^2 = 0.99$; $n = 20$).

able from unlabeled beetles in the population for up to 4.5 d postemergence. However, the mean Rb concentration of newly emerged beetles under laboratory conditions (2,772 ppm) was considerably greater than the highest mean Rb concentration obtained in the field (427 ppm for the soil + whorl treatment). If this difference in initial Rb concentrations were accounted for, based on the elimination curve, labeled beetles would remain distinguishable from unlabeled beetles for up to 3.2 d postemergence in the field.

A second laboratory study was conducted to determine if the Rb concentration in newly emerged beetles, previously labeled as larvae, could be elevated if the adults fed on Rb-enriched corn seedlings. Rb uptake rates for labeled males and females were not significantly different ($F = 0.19$; $df = 1, 33$; $P > 0.05$), so a single uptake curve was fitted to data from both sexes (Fig. 3A). After 1 d of feeding on Rb-enriched corn seedlings, the average Rb concentration in previously labeled beetles increased by 70% (Fig. 3A). There was a 5.5-fold increase in the Rb concentration of labeled beetles after feeding for 3 d (Fig. 3A). Although this experiment was conducted in the laboratory with seedling corn, these data suggest that the initial Rb concentration in beetles on emergence would be enhanced by feeding on labeled corn plants in the vicinity of their emergence site. Elevated initial Rb concentrations could then potentially extend the time-period beetles remain labeled in the field.

Rb concentrations in newly emerged, unlabeled beetles increased asymptotically with time after feeding on Rb-enriched corn seedlings (Fig. 3B). Rb uptake was significantly greater for females compared with males ($F = 9.51$; $df = 1, 41$; $P < 0.01$), and separate uptake curves were developed for each sex. Assuming a baseline Rb concentration of 5 ppm (2001 field study), unlabeled male and female beetles would acquire sufficient Rb to be labeled after <1 min of feeding on Rb-enriched corn seedlings. If the mean Rb concentration of newly emerged, labeled beetles reared in the laboratory is used as a benchmark

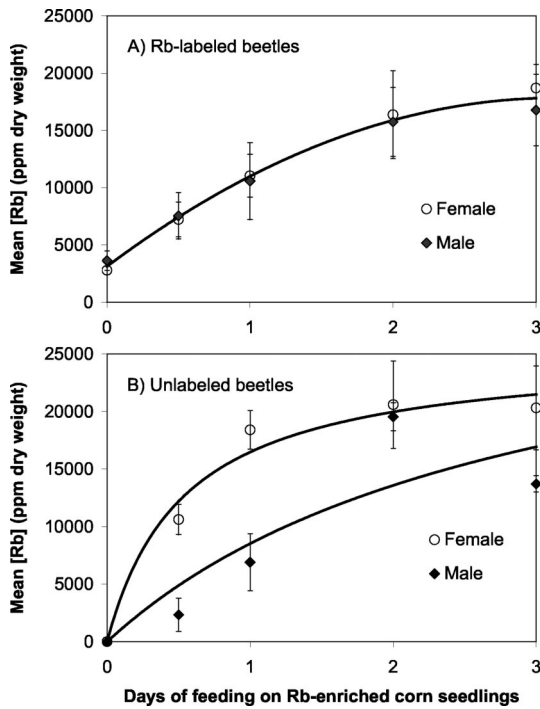


Fig. 3. Rb uptake in newly emerged western corn rootworm adults reared as larva on either (A) Rb-treated corn seedlings or (B) untreated corn seedlings, and caged on Rb-enriched corn seedlings for up to 3 d postemergence. Rb uptake in labeled beetles (A) followed a quadratic polynomial: $y = 3,121 + (9,360 * x) + (-1,488 * x^2)$; ($R^2 = 0.99$; $n = 8$). Uptake in unlabeled beetles (B) followed a rectangular hyperbola for males: $y = 33,399 * x / (2.92 + x)$; ($R^2 = 0.79$; $n = 4$); and females: $y = 25,336 * x / (0.54 + x)$; ($R^2 = 0.97$; $n = 4$).

(2,882 ppm), unlabeled male and female beetles would acquire equivalent levels of Rb in 6.5 and 1.7 h of feeding on Rb-enriched corn seedlings, respectively.

Discussion

Our results are in agreement with previous studies that show Rb is highly mobile and readily translocated throughout the corn plant. However, most previous studies in corn have focused on foliar applications of Rb to label lepidopteran pests that feed as larvae on above-ground plant parts (Graham et al. 1978b, Legg and Chiang 1984, Stimmann 1991). These studies demonstrate the feasibility of enriching corn plants with Rb in the field, either through Rb application to the soil, whorl, or both, with subsequent transfer of the Rb to western corn rootworm adults that complete development as larvae feeding on the roots. Application of Rb in solution both to the soil around the base of the plant and directed into the whorl provided the highest concentration of Rb in newly emerged beetles compared with the other application methods tested. At least 90% of the beetles that emerged from this treat-

ment during the first 3 wk of the emergence period contained Rb levels above the baseline concentration calculated from the untreated beetles.

In both years, there were no significant differences in the dry weights of beetles that completed development on Rb-enriched corn compared with beetles from untreated corn. Previous studies with several lepidopteran species report no adverse fitness effects until Rb concentrations in their diet increased above 10,000 ppm (Van Steenwyk 1991). In this study, median Rb concentrations in the roots approached only one-half that level when Rb was applied both to the soil and the whorl of the plants. This suggests that some beetle fitness parameters (i.e., size) were not negatively impacted by exposure to Rb as larvae.

The permanence of the mark is another factor that must be considered when determining the feasibility of a marking technique for dispersal studies (Berry et al. 1972, Van Steenwyk 1991). Western corn rootworm beetles labeled with Rb in the field should remain distinguishable from unlabeled beetles for up to 3.2 d postemergence. This time period is considerably shorter than the 10–14 d reported by Fleischer and Kirk (1994) for a related species, the southern corn rootworm, *Diabrotica undecimpunctata howardi* (Barber). However, in their study, beetles were caged and provided a diet of Rb-treated cucurbit seedlings for 7 d (i.e., no-choice situation) before being transferred to clean cucurbit transplants. This resulted in initial Rb concentrations that were considerably higher ($12,789 \pm 458$ ppm) than concentrations determined for newly emerged western corn rootworms ($2,772 \pm 51$ ppm) and could explain the difference in Rb elimination rates between the two species. The primary advantage of the labeling method developed in the current study is that it ensures beetles are labeled at the time of their emergence from the soil. The technique does not require that newly emerged beetles feed to become labeled. This is especially important, given our ultimate objective of measuring the initial premating movement capabilities of beetles from their emergence site.

Previous studies have shown that primarily mated, preovipositional western corn rootworm females engage in dispersal (Godfrey and Turpin 1983, Coats et al. 1986, Naranjo 1990a, 1991). Quiring and Timmins (1990) concluded that most females mated within the first 24 h after emergence and observed that some females only moved within a few meters of their emergence site for mating. These observations, along with results from our laboratory experiments, do suggest that initial Rb concentrations could be significantly elevated if newly emerged beetles fed on Rb-enriched corn plants in the vicinity of their emergence site. After 1 d of feeding on Rb-enriched corn seedlings, the average Rb concentration in labeled beetles increased by 70%. Elevated initial Rb concentrations could then possibly extend the time-period beetles remain labeled in the field.

Only a single application of Rb was applied to the corn plants in both years of this study. Graham et al. (1978b) determined that by dividing the total Rb ap-

plied into three weekly applications, Rb concentrations in both the corn plants and corn earworm larvae, *Helicoverpa zea* (Boddie), were initially higher, and remained elevated significantly longer, compared with the same amount of Rb in a single treatment. This suggests that by dividing the total amount of Rb applied over two to three applications, it may be possible to not only increase Rb concentrations in newly emerged beetles, but also increase the proportion of late-emerging beetles that are labeled.

The relatively short retention time of Rb in western corn rootworm beetles does not preclude this marking technique as useful for studying adult dispersal. It does, however, define the constraints associated with this marking technique. Even though the retention-time of Rb is relatively short, the ability to efficiently label a large number of beetles with virtually no disruption of the population should allow this marking technique (application of Rb to both the soil and whorl of plants) to be useful for quantifying the short-range, intrafield movement of western corn rootworm beetles from their emergence site. Additionally, because beetle emergence can last at least 6 wk and Rb concentrations remain elevated in the corn plants for at least 8 wk, the short retention time of Rb in beetles will allow for repeated measurements of beetle movement throughout the season. Such information would reduce the uncertainty associated with the development of effective resistance management strategies for corn rootworms and transgenic corn. Quantifying parameters associated with corn rootworm movement within the cornfield also should be valuable in the development and validation of models used to help assess risk associated with the evolution of resistance in rootworm populations to new control technologies.

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