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ABSTRACT Experiments were undertaken to determine the potential for using rubidium chloride (RbCl) or cesium chloride (CsCl) to mark southwestern corn borer, *Diatraea grandiosella* Dyar, for use in applied ecological studies. Maize, *Zea mays* L., plants were sprayed with aqueous solutions of RbCl or CsCl at rates of 100, 1000, or 10,000 μg/g and inoculated with *D. grandiosella* neonates. Rubidium and cesium were successfully absorbed and translocated in maize plants. There were only a few minor effects of the treatment on maize or on southwestern corn borers. Rb and Cs were detected in plants, but not in insects, by using flame atomic absorption spectrophotometry. Graphite furnace-atomic absorption spectrophotometry (GF-AAS) and neutron activation analysis (NAA) allowed identification of Rb and Cs in adults. Rb and Cs were detected by GF-AAS in feral unmarked adults, and they contained higher levels of Rb than Cs. Males and females contained similar amounts of Rb, but Cs levels were higher in males than in females. Adults recovered from field maize treated with 1000 μg/g Cs had higher levels of Cs than did those from untreated plants. Using NAA, neither Rb nor Cs was detected in adults recovered from greenhouse-grown untreated maize. Males and females recovered from maize treated with 1000 μg/g RbCl and CsCl contained similar amounts of Rb, but females contained more Rb than Cs. We conclude that application of 1000 μg/g RbCl or CsCl on plants is effective in marking adults of *D. grandiosella* with Rb or Cs and would be useful for mark–recapture dispersal studies.

KEY WORDS elemental marking, corn, resistance management, southwestern corn borer, *Zea mays*

INFORMATION ON DISPERSAL IS critical for designing and evaluating management strategies for highly mobile insects (Turchin and Thoeny 1993). This information is particularly important in the development of models that can predict the evolution of resistance in insects to manage resistance, as required with transgenic crops (Ostlie et al. 1997).

European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar, are destructive stalk-boring pests of maize, *Zea mays* L., in the United States (Chippendale 1979, Hyde et al. 2003), and the transgenic *Bacillus thuringiensis* (Bt)-maize hybrids (Koziel et al. 1993) have been developed to protect against these pests. The European and southwestern corn borers occur sympatrically in many areas where Bt maize is grown. Both species have the potential to develop resistance to Bt-maize hybrids (Ostlie et al. 1997), and the Environmental Protection Agency currently requires the planting of a 20% refuge of non-Bt maize (Ostlie et al. 1997, U.S. EPA 2001) to manage resistance. For this strategy to work, the moths must disperse and mate randomly and extensively. Our understanding of corn borer moth dispersal is based on observations on the European corn borer in the central plains of North America (Showers et al. 2001) where conditions are relatively humid. On the semiarid western high plains of North America, the European and southwestern corn borers seem to spend more time in the irrigated maize fields than in surrounding vegetation, as they do further east (Hunt et al. 2001). Thus, additional studies are needed on adult dispersal of these corn borers in irrigated fields. Studies on dispersal usually require the use of a marker that can identify insects that have dispersed away from the release point. Several markers have been evaluated for use with the European

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corn borer, but they have not been evaluated for use with the southwestern corn borer.

Most markers require handling the insects in captivity, which may potentially alter the behavior of the insect. An effective marker should be easily applied and marked insects should be identifiable within the feral population. The marker should last long enough for the test, and it should not affect the insect's behavior, growth, reproduction, or life span. A good marker should allow the marked insects to become thoroughly mixed within the feral population (Hagler and Jackson 2001).

Rubidium (Rb) and cesium (Cs) have been used to mark a variety of insects, including Heteroptera, Diptera, Homoptera, Coleoptera, Orthoptera, Hymenoptera, and Lepidoptera (Hagler and Jackson 2001, Jost and Pitre 2002). Legg and Chiang (1984) evaluated the use of Rb as a marker for European corn borer. Salts of these elements can be applied to plants as an aqueous spray solution (Berry et al. 1972, Moss and Van Steenwyk 1982), and insects that feed on these plants accumulate elevated levels of these rare elements. The marked insects can then be identified by screening insects for higher than normal concentrations of these elements. This allows the insects to develop in their natural habitat and avoid the problems associated with using laboratory-reared insects or handling field-collected insects (Prasifka et al. 2001, Jost and Pitre 2002). Background levels of Rb and Cs are very low, and these elements are relatively nontoxic because they mimic potassium, which is present in reasonable concentrations in plant and animal tissues (Berry et al. 1972, Moss and Van Steenwyk 1982, Jost and Pitre 2002). Availability of more than one marker allows their use in studies where there are multiple release sites or multiple release times. Whereas both Rb and Cs have been used to mark various insects, their effects on additional host plants and insects need to be determined (Moss and Van Steenwyk 1982, Jost and Pitre 2002).

Rb and Cs in plant and insect tissues are usually measured using atomic absorption spectrophotometry (AAS) (Moss and Van Steenwyk 1982, Prasifka et al. 2001, Jost and Pitre 2002), and less frequently using neutron activation analysis (NAA) (Monro 1968, Costa and Byrne 1988). Different measurement techniques vary in sensitivity and thus may be useful for different types of studies.

In the current study, we determined the amount of RbCl and CsCl needed to treat maize plants to mark southwestern corn borer adults that developed on sprayed plants and whether there were deleterious effects of RbCl and CsCl treatments on plants or southwestern corn borer.

Materials and Methods

2000 Greenhouse Experiment

This study was conducted from February through April at the Kansas State University Southwest Research-Extension Center (SWREC), Garden City, KS. Maize plants were grown in the greenhouse with additional light provided by 500-W halogen lamps with a photoperiod of 16:8 (L:D) h. Maize seed (NK 590 and Pioneer 3162IR) was planted at the rate of six seeds per 19 liter pot and later thinned to three plants per pot. Each pot was a replication, and there were four replications. NK 590 and Pioneer 3162IR were each planted in two of the four replications. There were seven treatments: 100, 1000, and 10,000 μg/g applications of RbCl and CsCl, and an untreated control.

Aqueous solutions of RbCl and CsCl (99% pure, Sigma, St. Louis, MO) were prepared in distilled water with 1% Triton B-1956 (Sigma) as surfactant. The test solutions were sprayed onto the plants with a handheld garden sprayer (Premium Lawn and Garden Sprayer, model 1000P, Gilmour, Somerset, PA) by using a hollow-cone nozzle when the plants were at the six- to eight-leaf stage (44 d after planting). Pots were rotated slowly on a turning plate (lazy susan) while the plants were sprayed for 50 s. This was calibrated to deliver 400 ml of solution per three plants (133 ml per plant) to reach a level for the solution to runoff. Control plants were sprayed with distilled water. After the plants had dried, they were inoculated with southwestern corn borer neonates in corncob grits by using a bazooka applicator (Davis and Oswalt 1979). Two to four neonates were added per day over a 5-d period until the total per plant reached 14. The neonates came from eggs obtained from a colony maintained on a wheat germ-based meridic diet (Davis 1976) at the SWREC. The colony had been established ≈6 mo earlier from local field-collected larvae.

Thirty six days after treatment, we recorded plant height, total number of leaves, and number of leaves showing phytotoxic symptoms. At the same time, we dissected the plants to collect the larvae and pupae and record insect damage. The number of tunnels was recorded and tunnel length measured per plant to evaluate larval behavior. Insects were held individually in 2-oz (59-ml) plastic rearing cups for development to pupation and adult eclosion. Cubes of the meridic southwestern corn borer diet with aureomycin and formaldehyde as antibiotics (Chippendale 1972) were added to the cups as needed to provide nutrition for developing larvae and humidity for both life stages through eclosion. The pupae were weighed within 24 h of pupation. The dates of pupation and adult eclosion were noted, and the numbers of eclosed and deformed adults were recorded. Adults were frozen at −20°C for later processing.

Plant tissues were collected from one plant from each pot (replicate) to determine Rb and Cs concentrations. These tissue samples were collected 36 d after the application of RbCl and CsCl, at the time when the plants were dissected to collect the larvae and pupae. A leaf blade and a stem section were collected from the lower part of the plant, which was exposed to the spray applications, and designated lower leaf (LL) and lower stem (LS). Another leaf blade and stem section were collected from the upper part of the plant, which

developed after the spray applications were made, and designated upper leaf (UL) and upper stem (US). Additional tissues also not directly exposed to spray applications were collected: roots (R), ear (E), and tassel (T). In some cases, plants were stunted by southwestern corn borer feeding so ears and tassels did not develop and were not available for sampling. Plant tissues were washed twice with tap water and then rinsed with distilled water to remove any unbound Rb and Cs on the surface. The washed plant tissues were allowed to air dry before they were packed in paper bags and frozen at −20°C for later processing.

2001 Greenhouse Experiment

The experiment was repeated in 2001 with some modification. This study was conducted from January through March in the greenhouse at Kansas State University, Manhattan, KS. This experiment included only the 1000 μg/g treatment of RbCl or CsCl and a single maize hybrid (Pioneer 3162IR). The plants were inoculated with southwestern corn borer neonates by using a soft camel's-hair brush rather than the bazooka.

Field Experiment

Pioneer 3163IR field maize was planted on 12 May 2000 at the SWREC. A plot of seven rows by 50 feet (≈500 total plants) was sprayed with 1000 μg/g CsCl in tap water with 1% Triton B-1956. The treatments were applied on 29 June 2000 when the plants were in the 12- to 14-leaf stage. A backpack sprayer (Swissmex, Sp1-E, Forestry Suppliers Inc., Jackson, MS) fitted with a cone nozzle was used to apply the solution. Each plant row (75 plants) was sprayed with a measured 6.4 liters of the solution (85 ml per plant) consistently across all seven rows. The application was timed to take three passes on each row, two passes with the nozzle directed at each side of the plants, and a third pass with the nozzle directed over the top of the plants. After the plants had dried, they were inoculated with a total of 40 southwestern corn borer neonates per plant in corncob grits by using the bazooka applicator. These southwestern corn borer neonates were supplied by the Monsanto Co., Chesterfield, MO. This population was derived from a colony established 1 yr earlier from larvae collected in Mississippi and/or Tennessee.

Feral adults were collected in light traps during the first generation flight of southwestern corn borer. These adults were collected before the experimental plants were treated and therefore had emerged from untreated maize. Ten plants from the CsCl-treated plants were dissected 35 d after they were treated to collect the pupae (all larvae had pupated). The pupae were kept in 2-oz (59-ml) plastic rearing cups with cubes of the meridic southwestern corn borer diet to provide humidity until adult eclosion. All adults were frozen at −20°C for later processing to detect Rb and Cs. The feral adults were analyzed to determine background concentrations of Rb and Cs. The Cs concentrations in feral adults and the adults recovered from CsCl-treated corn were compared.

Detection of Rb and Cs in Plants and Adults

Flame-Atomic Absorption Spectrophotometry (F-AAS). Plant and insect tissue samples from the 2000 greenhouse study were analyzed for Rb and Cs by using an air-acetylene oxidizing flame of an F-AAS (AAnalyst 100, PerkinElmer Life and Analytical Sciences, Boston, MA) following the modified procedures of Moss and Van Steenwyk (1982). Plant tissue samples were oven dried at 55°C for 5 d, powdered, and a 0.15-g subsample was added to a glass vial with Teflon-lined screw cap, along with 1.0 ml of acid mixture (90% HNO_3 and 10% H_2SO_4). The mixture was allowed to digest in the oven (Precision Thelco, model 18, Precision Scientific Co., Chicago, IL) at 120°C for 1 h. The contents were cooled, and the volume was brought to 5.0 ml by adding 3.0 ml of deionized water and 1.0 ml of KCl as an ion suppressant (Legg and Chiang 1984). Insect samples were oven dried at 95°C for 48 h and digested as described for plant tissues. Because Rb and Cs concentrations were very low, the samples were "spiked" by adding 0.5 ml of deionized water containing 20 μg/g Rb and Cs. Then, 0.3 ml of KCl was added to bring the volume to 1.8 ml. The electrodeless discharge lamps were set to wavelengths of 780 and 852.1 nm to detect Rb and Cs, respectively. For each element, the spectrophotometer was set to record three absorbance readings per sample at a reading time of 0.5 s. Standard Rb and Cs solutions of 1, 2, 4, 6, 10, and 15 μg/g were prepared and analyzed to calibrate the instrument. In cases where absorbance readings exceeded standard limits, the samples were diluted and read again. The spiked samples were evaluated for differences in concentrations between treated and untreated adults.

Graphite Furnace-Atomic Absorption Spectrophotometry (GF-AAS). The adults that emerged from the pupae collected from the treated plants and the feral adults were analyzed for Rb and Cs by using GF-AAS (AAnalyst 800, PerkinElmer Life and Analytical Sciences). Insects were oven dried individually at 60°C for 4 d and digested in 500 μl of nitric acid (70%) at 80°C for 1 h. Twenty microliters of the digested solution was added to 680 μl of nanopure water (35× dilution) to bring the final HNO_3 concentration to 2% for injection onto the GF-AAS. Electrodeless discharge lamps were set at wavelengths of 780 and 852.1 nm to analyze for Rb and Cs, respectively. Standard solutions of 1, 5, 10, and 20 ng/g Rb and 1, 2, 5, and 10 ng/g Cs were analyzed to calibrate the instrument. The conditions were set as specified for the AA 800 (PerkinElmer 1995), except that read time was set at 4 s rather than 3 s.

NAA. Adults from the 2001 greenhouse study were analyzed for Rb and Cs by using NAA (TRIGA MK-II, General Atomics, San Jose, CA). No protocols were available for detecting Rb and Cs by NAA; therefore, protocols were developed as part of this study.

Insects were dried for 48 h in a 2-liter bell jar by using a desiccant. National Institute of Standards Technology (NIST) Standard Reference Materials (SRM) were maintained in a similar bell jar with desiccant. Standard Reference Materials 1632a (trace elements in coal) and 1633a (trace elements in coal fly ash) were selected to support comparative neutron activation analysis with NIST-certified concentrations of Rb and Cs in SRM1632a, and reference values for SRM1633a.

Each adult was weighed and packaged in 1.5-ml polyethylene sample vial. Six sets of SRM standards were similarly prepared for each irradiation. Each sample vial was packaged in an outer container, a resealable plastic 2.54 by 2.54-cm polyethylene bag. Packaged samples and standards were loaded in sets of six into polyethylene vials (2.54 cm in diameter by 10.16 cm in height). Approximately 100 samples and standards were placed in the reactor for each irradiation; vials containing specimens and standards were placed in the rotary specimen rack (RSR) by using positions 1–18 (40 possible positions) as required. The reactor was operated at a power level corresponding to 1×10^{12} n/cm^2-s for 8 h. To ensure uniform irradiation, the RSR was rotated one-quarter turn every 0.5 h.

After irradiation, vials were removed from the RSR and allowed to decay for 1 to 4 mo so that isotopes with short half-lives would not interfere with the analysis. The outer bag covering each sample and standard bag was removed and replaced with new, unirradiated (nonradioactive) bags to reduce radioactive contamination. The radiation characteristics of Rb and Cs were measured for each sample and standard for a 1-h counting period in the solid-state semiconductor radiation detector (40% high-purity germanium) (Canberra Industries, Meridian, CT). The known concentrations and measured activity of Rb and Cs in the standards were compared with the measured activity in the samples to determine concentration in the sample by using the Genie computer software (Genie 2000). All Rb and Cs data for plants or insects are presented as micrograms per gram on a dry matter basis.

Statistical Analysis

With the 2000 greenhouse evaluations, percentage of survival of southwestern corn borer at plant dissection was calculated as the number of larvae and pupae found in the plants divided by the number of neonates placed on the plants at inoculation. Percentage of survival at adult eclosion was calculated as the number of adults eclosed divided by the number of neonates. Larval period was the number of days from inoculation to pupation. Larval and pupal periods were analyzed only when larvae pupated on the diet (pupation dates were known). Pupal weight (milligrams) included pupae that pupated in the plants and those that pupated on the meridic diet. Data for males and females were analyzed separately for larval period, pupal period, pupal weight, and adult dry weight. Percentage of pupation rate at plant dissection was calculated as the number of pupae at plant dissection divided by the number of neonates. Number of pupae at plant dissection was then combined with those that developed on the meridic diet to determine the overall pupation rate. Adult eclosion from pupae was calculated as the number of adults that emerged divided by the number of total pupae in each treatment. Deformity of eclosed adults was calculated as the number of deformed adults divided by the total number of eclosed adults.

The 2000 greenhouse data for the effects of elements on plants and southwestern corn borer were analyzed as nonparametric one-way analysis of variance (ANOVA) with ranked transformations (Conover 1999) by using PROC GLM (SAS Institute 1999–2000). This analysis was chosen after evaluating residuals for approximate normality by using residual stem and leaf plots. Mean separations were done using the Student–Newman–Keuls test ($P = 0.05$) by using ranked transformations (Zar 1999). The Student–Newman–Keuls test was used because there were more than four means in the test.

Rb and Cs concentrations in the plants were analyzed as a split plot (pot), two-factor (treatment and tissue) ANOVA with correlated subplots by using PROC MIXED. The Satterthwaite option was used to calculate degrees of freedom in PROC MIXED, so some degrees of freedom reported are not whole numbers. If interaction between treatment and tissue was significant, then tissues were analyzed within treatments for differences in Rb or Cs using one-way ANOVA, and means were separated with Student–Newman–Keuls procedure. The relationship between Rb and Cs in different plant tissues and application rates of RbCl and CsCl was analyzed using TableCurve 2D software (Jandel Scientific 1996). The software calculates a number of parameters for the candidate model equations including intercept, slope, R^2, maximum attainable R^2, and also tests the model for lack of fit. A good fit should have an R^2 that is close to the maximum attainable R^2 and no significant lack of fit.

Rb and Cs in adults from the 2001 greenhouse study were analyzed as nonparametric one-way ANOVA by using PROC GLM. Differences in treatment means were analyzed for separation using least significant difference (LSD) procedure ($P = 0.05$). LSD was used in analysis because there were four or fewer treatment means.

Rb and Cs in adults from the 2000 field experiment and feral male and female adults were analyzed as nonparametric one-way ANOVA by using PROC GLM. Differences in treatment means were analyzed for separation by using LSD ($P = 0.05$).

Whereas the statistical analyses were conducted on ranked transformed data, the means have been back-transformed into the original units to simplify interpretation.

Results

Effects of RbCl and CsCl on Plants. Only the 10,000 μg/g CsCl treatment in the greenhouse had significantly more leaves (mean ± SE, 5.25 ± 0.52) with phytotoxic symptoms than the other treatments

Table 1. Effects (mean ± SE) of 100, 1000, and 10,000 μg/g RbCl and CsCl treatments on southwestern corn borer development on treated plants during 2000 greenhouse experiment

Treatment	Survival (%)		Larval period (d)		Tunnels per plant		Pupal period (d)	
	at PD[a]	at AE[b]	Male	Female	Number	Length (cm)	Male	Female
Control	20.24 ± 2.86	11.31 ± 2.47	64.00 ± 6.38	58.64 ± 3.30b	3.75 ± 0.42ab	51.08 ± 3.80b	11.40 ± 1.12	10.42 ± 0.49
RbCl 100	23.81 ± 2.86	17.26 ± 2.47	52.80 ± 3.68	43.00 ± 2.92a	3.91 ± 0.42ab	47.92 ± 3.80b	11.00 ± 0.80	11.14 ± 0.64
RbCl 1000	16.67 ± 2.86	13.09 ± 2.47	56.62 ± 5.04	46.10 ± 3.03ab	3.25 ± 0.42a	45.59 ± 3.80ab	13.00 ± 1.12	11.25 ± 0.60
RbCl 10000	13.09 ± 2.86	9.52 ± 2.47	48.60 ± 6.38	49.63 ± 3.29ab	3.00 ± 0.42a	33.33 ± 3.80a	14.00 ± 1.80	11.92 ± 0.49
CsCl 100	21.43 ± 2.86	16.67 ± 2.47	59.28 ± 3.81	54.92 ± 3.03b	4.17 ± 0.42ab	48.84 ± 3.80b	12.62 ± 0.70	11.63 ± 0.51
CsCl 1000	23.21 ± 2.86	15.48 ± 2.47	56.37 ± 5.04	52.81 ± 2.73b	5.08 ± 0.42b	56.59 ± 3.80b	13.60 ± 0.95	12.56 ± 0.42
CsCl 10000	17.26 ± 2.86	10.71 ± 2.47	59.71 ± 5.04	57.33 ± 3.16b	3.00 ± 0.42a	40.59 ± 3.80ab	11.80 ± 1.03	11.73 ± 0.51
df	6, 21	6, 21	6, 19	6, 21	6, 21	6, 21	6, 15	6, 20
F	2.1	1.7	1.1	4.8	3.2	4.2	2.0	2.1
P	0.100	0.178	0.402	0.003	0.022	0.007	0.130	0.093

Treatment	Pupation (%)		Pupal weight (mg)		Adults (%)		Adults dry weight (mg)	
	at PD[a]	Overall[c]	Male	Female	Eclosed[d]	Deformed[e]	Male	Female
Control	4.76 ± 1.26ab	18.45 ± 2.91	168.40 ± 16.67	267.73 ± 14.99	70.09 ± 6.81	8.57 ± 11.21	40.33 ± 5.51	56.98 ± 5.72
RbCl 100	10.12 ± 1.26b	22.02 ± 2.91	165.07 ± 9.62	239.21 ± 13.28	79.05 ± 6.81	21.88 ± 11.21	32.57 ± 3.48	48.71 ± 5.26
RbCl 1000	4.76 ± 1.26ab	15.48 ± 2.91	168.50 ± 13.18	226.23 ± 13.79	85.04 ± 6.81	17.78 ± 11.21	32.60 ± 4.77	55.91 ± 5.26
RbCl 10000	4.17 ± 1.26ab	10.71 ± 2.91	157.00 ± 16.67	221.91 ± 14.99	91.43 ± 6.81	4.17 ± 11.21	28.48 ± 6.03	53.89 ± 5.72
CsCl 100	1.79 ± 1.26a	20.83 ± 2.91	176.86 ± 9.96	221.08 ± 13.79	79.91 ± 6.81	13.57 ± 11.21	35.99 ± 3.48	53.12 ± 5.26
CsCl 1000	2.98 ± 1.26ab	23.21 ± 2.91	185.00 ± 13.18	246.75 ± 12.42	67.15 ± 6.81	19.29 ± 11.21	34.11 ± 4.77	57.61 ± 4.47
CsCl 10000	1.79 ± 1.26a	15.48 ± 2.91	176.57 ± 14.09	229.17 ± 14.35	69.72 ± 6.81	35.00 ± 11.21	37.60 ± 5.10	52.63 ± 5.72
df	6, 21	6, 21	6, 19	6, 21	6, 21	6, 21	6, 19	6, 21
F	3.2	2.4	0.4	2.3	2.0	0.6	0.6	0.8
P	0.023	0.063	0.895	0.073	0.106	0.732	0.747	0.584

Means within a column bearing the same letter or no letter were not significantly different ($P > 0.05$, Student–Newman–Keuls, PROC GLM, LS MEANS).

[a] Survival or pupation at plant dissection (PD) calculated against the number of neonates inoculated.

[b] Adult eclosion (AE) from the pupae calculated against the number of neonates inoculated.

[c] Total pupation on plants and diet calculated against the number of neonates inoculated.

[d] Adult eclosion calculated against the available pupae.

[e] Deformed adults calculated against the eclosed adults.

(range 0–1.25 ± 0.52) ($F = 8.6$; df = 6, 21; $P = 0.001$). The effects on plant height were significant in the ANOVA ($F = 3.0$; df = 6, 21; $P = 0.030$), but the means were not significantly different across treatments in the Student–Newman–Keuls rankings. Mean (± 16.92) plant height was 80.50 cm in control treatment; 90, 113.75, and 150.50 cm in 100, 1000, and 10,000 μg/g RbCl treatments, respectively; and 128, 110.25, and 151.50 cm in 100, 1000, and 10,000 μg/g CsCl treatments, respectively.

Effects of RbCl and CsCl on Southwestern Corn Borer. Rubidium and cesium had little effect on southwestern corn borers (Table 1). There were no differences among treatments in the greenhouse for survival at plant dissection or adult eclosion, male larval period, pupal period for males or females, overall percentage pupation from plants and diet, weights of pupae and males and females, and overall percentage of adults that eclosed from pupae and deformed adults. Female larvae developed faster in the 100 μg/g RbCl treatment than in the control the three CsCl treatments (Table 1). There were fewer tunnels per plant in 1000 and 10,000 μg/g RbCl treatments, and in 10,000 μg/g CsCl treatment, than in the 1000 μg/g CsCl treatment (Table 1). There was less tunneling in 10,000 μg/g RbCl treatment than in the control, 100 and 1000 μg/g CsCl treatments, and 100 μg/g RbCl treatment (Table 1). Percentage of pupation in the six treatments of RbCl and CsCl did not differ from the control at plant dissection, but it was higher in the 100 μg/g RbCl treatment than in the 100 and 10,000 μg/g CsCl treatments (Table 1).

Absorption and Translocation of Rb and Cs in Plants. Mean plant Rb averaged across six tissues differed among RbCl treatments in the greenhouse ($F = 182.2$; df = 3, 60.1; $P < 0.001$) (Fig. 1); however, it did not differ among plant tissues ($F = 1.1$; df = 5, 60; $P = 0.347$), and there was no interaction between treatment and plant tissue ($F = 1.1$; df = 15, 60; $P = 0.397$). The background concentration of Rb in the six tissues from control plants ranged between 0 and 4 μg/g. Relative to the control plants, Rb was not significantly higher in the 100 μg/g RbCl treatment ($t = 0.1$, df = 61.6, $P = 0.897$), but it was higher in the 1000 and 10,000 μg/g RbCl treatments ($t = 2.5$, 19.80; df = 59.7, 60; $P = 0.006$, 0.001, respectively) (Fig. 1). Rubidium was absorbed into plant tissues that were directly exposed to the spray treatments (lower stem and lower leaf, Fig. 2C and E). It also was translocated to the other plant tissues that were not exposed to the spray treatments or that developed later (roots, upper leaf, upper stem, and ear/tassel, Fig. 2A, B, D, and F).

Mean plant Cs averaged across six tissues differed among CsCl treatments ($F = 598.0$; df = 3, 62.3; $P = 0.001$) (Fig. 1), and it also differed among plant tissues ($F = 13.9$; df = 5, 62.5; $P = 0.001$). There was interaction between treatment and plant tissue ($F = 10.9$; df = 15, 62.4; $P = 0.001$), but these differences were

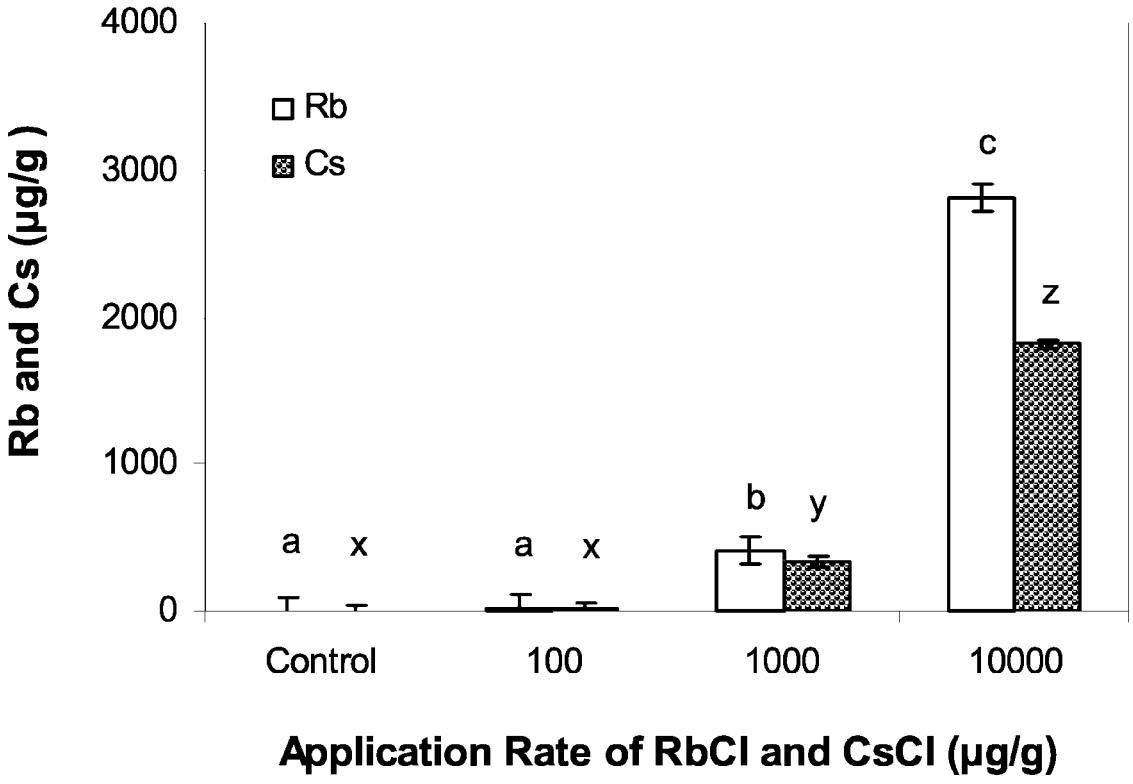

Fig. 1. Mean (±SE) rubidium and cesium content of plants (averaged over the six tissues) sprayed with different rates of RbCl and CsCl (Rb and Cs detected using F-AAS). Means with same letter in a, b, c series for Rb or x, y, z series for Cs are not significantly different across treatments (PROC MIXED, LS MEANS, $P > 0.05$).

small. The background concentration of Cs in the six tissues from control plants ranged between 2 and 13 μg/g. Relative to control plants, Cs was not higher in the 100 μg/g CsCl treatment ($t = 0.4$; df = 62.5; $P = 0.709$), but it was higher in the 1000 and 10,000 μg/g CsCl treatments ($t = 6.6$, 35.7; df = 62.2, 62.2; $P = 0.001$, 0.001, respectively) (Fig. 1). Cesium was absorbed into plant tissues that were directly exposed to the CsCl treatments (lower leaf and lower stem, Fig. 2C and E), and it also was translocated to the other plant tissues that were not exposed to the treatment or developed later (roots, upper leaf, upper stem, and ear/tassel, Fig. 2A, B, D, and F). However, in the 10,000 μg/g CsCl treatment, Cs was higher in the lower stem (1656.25 ± 158.71 μg/g) and lower leaf (2365.25 ± 158.71 μg/g) than in the upper stem (1142.75 ± 158.71 μg/g) ($F = 12.9$; df = 5, 17; $P = 0.001$).

The relationship between Rb and Cs in the plant tissues and the application rates of RbCl and CsCl can be explained by a linear regression model, $y = \beta_0 + \beta_1 (x)$, fit for each plant tissue, with β_0 representing the intercept, β_1 the slope, y the Rb or Cs in the plant tissue, and x the application rate of RbCl or CsCl in the aqueous solution. The intercept was not significantly different from zero for any of the regression equations ($P > 0.1$) (Fig. 2A–F).

Rb and Cs in Adults. F-AAS was not able to detect Rb and Cs reliably in the adult samples from the 2000 greenhouse study. The experiment was therefore repeated in 2001 to obtain more adults for further testing.

In total, 69 adults were available from the 2001 greenhouse experiment for Rb and Cs analysis by using NAA. Rubidium content was not different between males and females ($P = 0.476$) (Table 2), but females had more Rb than Cs ($P = 0.001$) (Table 2). There was only one male tested for Cs, so it was not included in the statistical analysis. Adults from the control plants did not have enough Rb and/or Cs to exceed the detection limit and were reported as zero (Table 2). All adults from the RbCl- and CsCl-treated plants in which Rb and Cs were reliably detected above the threshold could be considered "marked" with a confidence of >90% (Genie 2000). Seventeen of 25 adults (68%) from the RbCl-treated plants had enough Rb to be identified as marked, six of 13 males (46.15%) and 11 of 12 females (91.67%). Nine of 15 adults (60%) from the CsCl-treated plants had enough Cs to be identified as marked, one of six males (16.67%) and eight of nine females (89%).

Twenty feral adults from the field experiment were processed for Rb and Cs by using GF-AAS, 10 males and 10 females. Both males and females had more Rb

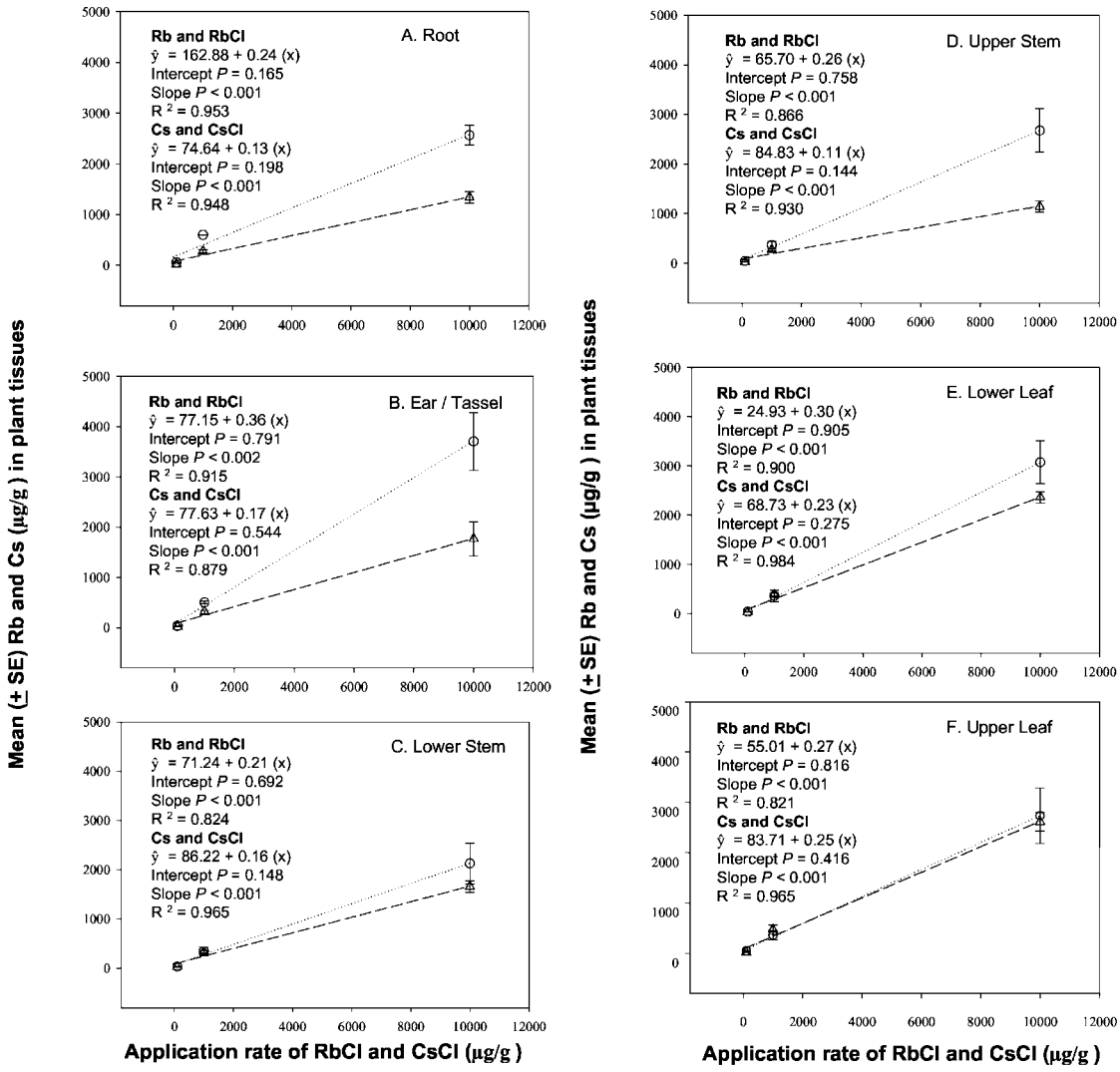

Fig. 2. Linear relationship [$y = \beta_0 + \beta_1 (x)$] between application rates (100, 1000, and 10,000 μg/g) of RbCl and CsCl sprayed on maize plants and the quantity of Rb and Cs detected in plant tissues by using F-AAS (···o···Rb and RbCl; ---^---Cs and CsCl).

than Cs ($P = 0.020, 0.001$) (Table 3). Rubidium content was not different for males and females ($P = 0.380$); however, males had more Cs than did females ($P = 0.001$). Rubidium was detected in all adults analyzed. Cesium was detected in all males, but three of seven females did not have detectable Cs.

In total, nine males and seven females recovered from dissected field CsCl-treated maize plants were processed for Cs by using GF-AAS. One female sample did not digest normally and had to be excluded. Cesium in adults recovered from CsCl-treated maize was significantly higher than Cs of feral adults from untreated maize (Table 4). If we set a threshold for Cs detection as the Cs mean for insects from untreated maize + 3 SD, then the threshold would be 0.222 μg/g for males and 0.048 μg/g for females. By using these as thresholds of detection, seven of nine males (77.78%) and all the females (100%) from the CsCl-treated plants could be identified as marked with Cs.

Discussion

Effects of RbCl and CsCl on Plants. There were only minor effects on plants that were treated with RbCl and CsCl, but the 10,000 μg/g CsCl treatment produced more leaf phytotoxicity than did the other treatments. The salts were applied with surfactant, which may have exacerbated the phytotoxic effects. In a subsequent trial the phytotoxic response was much worse when Triton 100 was used instead of Triton B-1956 (when the latter was no longer available; data not presented). The phytotoxic effects were associated with spray runoff that accumulated in the plant whorl. In summarizing the literature on plant effects

Table 2. Rubidium and cesium (micrograms per gram) (mean ± SE) of male and female adults of southwestern corn borer from plants sprayed with 1000 ppm RbCl or CsCl aqueous solutions in the greenhouse, 2001 (Rb and/or Cs detected using NAA)

Treatment	Gender	Insects processed	Rb (μg/g)				Cs (μg/g)			
			No. Rb detected	Mean	± SE	Range	No. Cs detected	Mean	± SE	Range
RbCl	Male	13	6	45.36a	10.59	20.05–61.06	0	0		
	Female	12	11	53.56a	7.80	10.79–117.56	0	0		
CsCl	Male	6	0	0			1	0.72[a]		
	Female	9	0	0			8	2.33b	9.14	0.61–3.87
Control	Male	11	0	0			0	0		
	Female	18	0	0			0	0		

One-way ANOVA: $F = 13.2$; df = 2, 8; $P = 0.003$. Means in both columns followed by the same letter are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).

[a] This single observation was not included in the analysis.

of Rb, Berry et al. (1972) stated that Rb was not toxic to plants even at high application rates.

Effects of RbCl and CsCl on Southwestern Corn Borer. The application of the two salts on maize had very minor effects on southwestern corn borer. Only female larval period and tunnel length were affected by some of the treatments. Female larval period was shorter in the 100 μg/g RbCl treatment than in the control. Tunnels per plant were not different between control and any of the RbCl or CsCl treatments; however, tunnel length in the highest rate of RbCl was lower compared with control. The latter is probably a function of survival because the number of tunnels per plant and tunnel length per plant followed the survival trends observed at the time of plant dissection. Previous studies with phytophagous Lepidoptera have not reported deleterious effects of RbCl and CsCl on insect development, longevity, behavior, mating, fecundity, or fertility (Van Steenwyk 1991).

Absorption and Translocation of Rb and Cs in Plants. Both Rb and Cs were absorbed and translocated within maize plants as evidenced by their occurrence in similar concentrations in plant tissues that were exposed or unexposed to the RbCl and CsCl treatments. Generally, there was less Cs in plant tissues than Rb, and this was most obvious at the 10,000 μg/g rate. It is unclear whether this is the result of differences in plant physiological responses to Rb and Cs or the result of Cs having higher atomic weight, so fewer atoms of Cs were applied to the plants. At the 100 and 10,000 μg/g application rates, there was 11–16% more Rb than Cs in the lower leaf and lower stem. The salt concentrations in the plants may have been higher immediately after treatment, but in this experiment the elements had 30 d to translocate to other parts of the plant before we measured them.

Rubidium seems to be more mobile than Cs because it did not occur in higher concentrations in exposed tissues than in unexposed tissues. This finding is in agreement with that of Wallace (1968), but not that of Bukovac and Wittwer (1957). Cesium seems to be less mobile than Rb because it occurred in higher concentrations in the exposed lower stem and leaf than in the unexposed upper stem in the 10,000 μg/g CsCl treatment. Levi (1970) reported higher Cs retention and lower levels of translocation for Cs than for Rb. However, Moss and Van Steenwyk (1982) found no difference in the Cs of cotton plant foliage exposed to 1.24 and 2.47 kg CsCl/ha spray and new foliage that grew after the applications had been made.

The relationship between levels of Rb and Cs in different plant tissues and the application rates of RbCl and CsCl can be described with a linear one-parameter (slope) model because intercept was not significant ($P > 0.1$). However, the linear model with two parameters (intercept and slope) was selected because there were detectable background levels of Rb and Cs in the untreated plants. Nonsignificant values for lack of model fit ($P > 0.05$) also support the linear relationship between Rb and Cs in the plant tissues and the application rate of RbCl and CsCl.

Rb and Cs in Adults. Feral males and females contained more Rb than Cs, but Cs was about eight-fold lower in feral females than in feral males, even though females are larger and probably consumed more plant tissue and would be expected to accumulate more of the elements. However, these females may have oviposited and passed much of their Cs to the eggs. However, males may have been unmated or may not pass on as much Cs in their ejaculate. However, females that developed in the laboratory on meridic diet containing Cs had almost double the Cs concentration that males had, even though the females had also deposited their eggs (Qureshi et al. 2004). Both Rb and Cs have been reported in feral populations and horizontal (mating) and vertical (oviposition) transmission has been demonstrated in other insects (Jost and

Table 3. Background levels of Rb and Cs (micrograms per gram) (mean ± SE) in feral male and female adults of southwestern corn borer collected at Garden City, KS, 2000 (Rb and/or Cs detected using GF-AAS)

Gender	Insects processed	Element	No. Rb or Cs detected	Concentration (μg/g)		
				Mean	± SE	Range
Male	10	Rb	10	0.141a	0.018	0.102–0.183
Female	10	Rb	10	0.187a	0.018	0.087–0.415
Male	10	Cs	10	0.107b	0.018	0.065–0.200
Female	10	Cs	7	0.014c	0.018	0.013–0.031

One-way ANOVA: $F = 25.2$; df = 3, 36; $P < 0.0001$. Means in the column followed by the same letter are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).

Table 4. Cesium content (micrograms per gram) (mean ± SE) of male and female adults of southwestern corn borer reared from CsCl-treated maize and feral adults from untreated maize, Garden City, KS, 2000 (Rb and/or Cs detected using GF-AAS)

Treatment	Gender	Insects processed	Concentration (μg/g)					No. exceeding threshold
			No. Cs detected	Mean	± SE	Range	Threshold = untreated Mean + 3 SD	
CsCl treated	Male	9	9	1.134a	0.427	0.107–7.830	0.222	7
	Female	6	6	0.891b	0.523	0.695–1.174	0.048	6
Untreated or feral	Male	10	10	0.107c	0.405	0.065–0.200		
	Female	10	7	0.014d	0.405	0.013–0.031		

One-way ANOVA: $F = 84.6$; df = 3, 31; $P < 0.0001$. All means are significantly different ($P < 0.05$, LSD, PROC GLM, LS MEANS).

Pitre 2002). The exact reasons for the transfer of these elements in various species needs further study. Pivnick and McNeil (1987) demonstrated that, at emergence, males of the *Thymelicus lineola* Ochsenheimer contained 2 to 3 times higher concentrations of abdominal sodium than did females and that males transferred 32% of that to females during the first mating. This was of considerable importance given that an average egg complement contains >50% of the total body sodium of females.

In insects that developed on field maize treated with 1000 μg/g CsCl, Cs concentrations were higher in males but were more consistent in females. Although variable, Cs-marked adults could be reliably differentiated from unmarked adults. The background levels of Rb and Cs in feral insects were extremely low with no significant overlap between marked and unmarked adults (mean background concentration + 3 SD).

In the greenhouse study, Rb concentrations were similar in male and female southwestern corn borer. This finding was similar to that of European corn borer reared on maize plants (Legg and Chiang 1984). However, Rb concentrations were different in males and females of the tobacco budworm, *Heliothis virescens* (F.) (Graham and Wolfenbarger 1977) and the pink bollworm, *Pectinophora gossypiella* (Saunders) (Moss and Van Steenwyk 1982).

Because southwestern corn borer adults have a short lifespan and do not feed, the likelihood of marker retention is greatly increased. However, other insect species were reported to lose marker with time after removal from the marked feeding source (Graham and Wolfenbarger 1977, Van Steenwyk et al. 1978). Significant retention of Rb in marked insects is largely dependent on the time spent feeding on the marked source versus time spent feeding on an unmarked source (Van Steenwyk 1991).

Graphite furnace-atomic absorption spectrophotometry and NAA were both effective in detecting Rb and Cs in adults. GF-AAS was more sensitive and was able to detect Rb and Cs in almost all the adults, including feral unmarked adults from the field. In contrast, NAA was not able to detect Rb or Cs in insects from untreated plants or in some insects from treated plants. The NAA depends on the mass of an element in a sample for detection. Single adult samples may not have enough mass of the element to be detected; however, when multiple samples are counted together, the probability of detection increases. That is why Rb and Cs were sometimes detected in samples of two to four adults together but not in the individual adult samples.

Time between irradiation and counting of gamma rays in the samples also can affect Rb and Cs detection. Exposure to the neutron flux irradiates a number of other elements, apart from the elements of interest (Rb and Cs). Energy from the radiation of those unwanted elements can interfere with the gamma ray counts for the target elements. The half-life of Cs is 2.062 yr and that of Rb is 18.631 d. The more time that elapses between irradiation and gamma ray counting, the more the short half-life elements decay and the better the chances of Cs detection. However, Rb samples need to be analyzed within 3 wk of irradiation. The chances of Rb detection are reduced due to the noise from the other short half-life elements. Costa and Byrne (1988) suggested that the relatively short half-life of some of the isotopes necessitate measurements being taken within a restricted period of time. Our results suggest that, both in the plants and in insects, Rb concentrations were generally higher than Cs concentrations regardless of the detection method.

Based on these studies, we conclude that the 1000-μg/g rate of RbCl and CsCl is appropriate for conducting dispersal studies in the field because there were only minor deleterious effects on plants or insects at this rate. Similar results also were recorded for southwestern corn borer feeding on meridic diets containing Rb and Cs (Qureshi et al. 2004). One application of 1000 μg/g RbCl or CsCl on greenhouse maize plants (133 ml per plant) or field maize (85 ml per plant) effectively marked southwestern corn borer that developed on the plants. Rb and Cs could be detected to differentiate insects from treated and untreated plants. Both the GF-AAS and NAA can be used to detect Rb and Cs in adults. GF-AAS seemed to be more sensitive than the NAA, but NAA may have enough sensitivity to detect markers for differentiating marked and unmarked adults from dispersal studies. A cost comparison of the two techniques was not available to us, because special arrangements were made to use the instruments. The choice of instrument will depend on local availability, cost, and convenience.

Detection of elevated levels of Rb and Cs in the different tissues of the maize plant means that these markers can be used with a number of other species that feed on the maize plant. Even the western corn

rootworm, *Diabrotica virgifera virgifera* LeConte, may be marked with this technique because the roots contained significant concentrations of Rb and Cs. Graham et al. (1978) were able to mark corn earworm and fall armyworm, *Spodoptera frugiperda* (J.E. Smith) with Rb sprayed on maize at 10 and 20 kg RbCl/ha. It may even be possible to conduct dispersal studies in maize on more than one species, European and southwestern corn borers, by using a single marker or by using different markers in one species at multiple release sites or times.

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