Evaluation of Rubidium Chloride and Cesium Chloride Incorporated in a Meridic Diet to Mark *Diatraea grandiosella* (Lepidoptera: Crambidae) for Dispersal Studies

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ABSTRACT Southwestern corn borers, *Diatraea grandiosella* Dyar, were reared on a control meridic diet and diets that incorporated rubidium chloride (RbCl) or cesium chloride (CsCl) at the rate of 1000 μg/g (1 g/liter of wet diet) to evaluate the effects on biology of *D. grandiosella* and to determine whether the resulting adults are marked with the rubidium (Rb) and cesium (Cs) physiological markers. The effects of RbCl and CsCl on survival, diet consumption, larval and pupal weight, developmental time, adult deformity, adult longevity, fecundity, and adult dry weight were generally minor and seldom reached statistically significant proportions. Males and females mated successfully when paired in different combinations across treatments. Graphite furnace atomic absorption spectrophotometer and neutron activation analysis were both effective in detecting Rb and Cs in male and female adults reared on RbCl and CsCl diets. Rb and Cs concentrations of males and females reared on RbCl and CsCl diets were above the background levels found in adults reared on the control diet. Rb and Cs can be used as physiological markers to mark *D. grandiosella* in dispersal experiments.

KEY WORDS southwestern corn borer, elemental marking, dispersal, Bt maize

INSECT DISPERSAL HAS BEEN studied to understand several ecological interactions. Recently, the importance of dispersal has been demonstrated in the development of resistance management strategies for corn borers in *Bacillus thuringiensis* (Bt) maize, *Zea mays* L. In an effective resistance management strategy, susceptible corn borer adults must be able to disperse from refuge plantings into Bt maize fields to mate with potentially resistant adults (Ostlie et al. 1997). Such mating would produce offspring that are incapable of surviving on Bt maize as long as resistance is a recessive trait.

European corn borer, *Ostrinia nubilalis* (Hübner), and southwestern corn borer, *Diatraea grandiosella* Dyar, are two important pests of maize in the United States (Chippendale 1979, Calvin and Van Duyn 1999, Knutson and Davis 1999, Hyde et al. 2003). Transgenic Bt maize hybrids are effective in suppressing both species. An understanding of the dispersal behavior of these borers is needed to evaluate the effectiveness of the high-dose refuge resistance management strategy. Dispersal studies are usually conducted as mark–release–recapture or mark–recapture experiments (Reynolds et al. 1997). Mark–release–recapture experiments generally involve captured or laboratory-reared insects that are marked in captivity and then released. Mark–recapture studies involve application of the marker to the insect in the natural habitat, usually by applying it to the host plants on which the insects develop.

To study dispersal behavior, effective markers will be needed. To be effective, the insects should be marked with a marker that will not affect the flight and fitness of the insect so that it behaves like feral counterparts in the field. The ideal marker should not hinder, irritate, or change the insect's behavior, growth, reproduction, or life span (Southwood 1969, Hagler and Jackson 2001).

Rubidium (Rb) and cesium (Cs) have been used to mark insects physiologically (Berry et al. 1972; Moss and Van Steenwyk 1982, 1984; Fernandes et al. 1997; Prasifka et al. 2001; Jost and Pitre 2002). Moss and Van Steenwyk (1984) pointed out that Rb and Cs could be used in dispersal experiments to give three (Rb, Cs, and Rb/Cs) distinct markers. The different markers can be used to identify insects released in adjacent areas, or they could be used to identify insects released

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at different times. Akey (1991) and Hagler and Jackson (2001) reviewed the uses, successes, and problems associated with RbCl and CsCl to mark insects. In biological systems, Rb and Cs are not very toxic 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). Physiological markers are not visible, but they can be detected with appropriate instrumentation. Rb and Cs are usually detected using an atomic absorption spectrophotometer (AAS). Rubidium chloride (RbCl) and cesium chloride (CsCl) can be incorporated in the insect's diet either in the laboratory or in the field by spraying it on the host plant (Berry et al. 1972, Moss and Steenwyk 1984). Typically, RbCl or CsCl have negligible effects on the environment, on the host plant, or on the marked insects, unless they are used at very high rates. However, explicit evaluations need to be conducted to ensure that the marker does not adversely affect a new test insect (Hagler and Jackson 2001). Legg and Chiang (1984) have reported on the use of Rb as a physiological marker for the European corn borer. They found no adverse effects of RbCl on the preadult mortality, adult form, and fecundity of this insect reared on artificial diet containing 100, 1000, and 10,000 μg/g RbCl. These markers have not been tested for use with the southwestern corn borer.

The current studies were conducted to determine the feasibility of using diet-incorporated RbCl and CsCl to mark southwestern corn borer with Rb and Cs. The objectives were to determine 1) whether RbCl or CsCl exert negative effects on the development of southwestern corn borer at the tested rates; and 2) whether Rb and Cs can be measured at concentrations above background in adults resulting from larvae reared on diets with incorporated RbCl and CsCl.

Materials and Methods

Insects and Rearing. The insects used in this study were obtained from a southwestern corn borer colony maintained at the Southwest Research and Extension Center (Kansas State University, Garden City, KS). The colony was established within 10 mo from larvae collected from local maize. The insects were reared on a wheat germ meridic diet (Davis 1976) with aureomycin and formaldehyde included as antibiotics (Chippendale 1972). Experimental insects were reared on three different diets: control or standard diet, RbCl diet, and CsCl diet. The RbCl diet incorporated 1 g of RbCl (99% pure, Sigma-Aldrich, St. Louis, MO) per liter of diet, and the CsCl diet incorporated 1 g of CsCl (99% pure, Sigma-Aldrich) per liter of diet. This 1000 μg/g RbCl or CsCl amounts to 700 μg/g Rb and 790 μg/g Cs per liter of the wet diet. The 1000 μg/g rate had been identified in greenhouse evaluations as a rate that caused minimum deleterious effects on plants but was high enough that southwestern corn borer that developed on treated plants had enough marker to be detected (Qureshi 2003).

Table 1. Percentage of survival (mean ± SE) of southwestern corn borer over different time intervals when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Mean % survival (no.)[a]						
	Neonate-5-d-old larvae	6-10-d-old larvae	11-15-d-old larvae	16-d-old larvae-pupae	Pupation rate	Neonate-adult eclosion	Adult eclosion from pupae
RbCl diet	96.67 ± 1.57 (90)	98.85 ± 0.94 (87)	98.81 ± 0.94 (86)	93.95 ± 3.21 (85)	83.33 ± 4.67 (90)	61.11 ± 10.16 (90)	80.00 ± 10.80 (77)
CsCl diet	96.67 ± 1.57 (90)	98.85 ± 0.94 (87)	100.00 ± 0.94 (86)	94.21 ± 3.21 (86)	85.56 ± 4.67 (90)	47.78 ± 10.16 (90)	55.43 ± 10.80 (77)
Control diet	100.00 ± 1.57 (90)	100.00 ± 0.94 (90)	97.78 ± 0.94 (90)	95.48 ± 3.21 (88)	85.56 ± 4.67 (90)	66.66 ± 10.16 (90)	71.22 ± 10.79 (75)
F (df = 2, 6)	1.74	0.50	1.09	0.06	0.52	0.36	0.92
P	0.253	0.630	0.396	0.941	0.617	0.712	0.447

Means in columns are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).

[a] Mean ± SE and (in parentheses) total number of larvae or pupae in the three replications.

Table 2. Total diet consumption (mean ± SEM) of male and female southwestern corn borer larvae over different time intervals when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Sex	n^a	Diet consumed (mg)/larva/time interval			
			Neonate-5 d	6–10 d	11–15 d	Total neonate-pupae
RbCl diet	Male	42	630.48 ± 97.97	1097.74 ± 105.24	2483.47 ± 305.83	4211.69 ± 304.12
RbCl diet	Female	13	655.57 ± 97.97	1061.11 ± 105.24	2911.11 ± 305.83	4627.78 ± 304.12
CsCl diet	Male	31	608.67 ± 97.97	943.59 ± 105.24	2157.44 ± 305.83	3709.91 ± 304.12
CsCl diet	Female	12	611.11 ± 97.97	890.74 ± 105.24	2307.41 ± 305.83	3809.26 ± 304.12
Control diet	Male	36	519.05 ± 97.97	942.86 ± 105.24	2345.24 ± 305.83	3807.14 ± 304.12
Control diet	Female	24	493.81 ± 97.97	1003.67 ± 105.24	2526.67 ± 305.83	4024.29 ± 304.12
F (df = 5, 12)			0.29	0.85	1.10	1.62
P			0.911	0.540	0.412	0.229

Means in columns are not significantly different ($P > 0.05$, Student–Newman–Keuls, PROC GLM, LS MEANS).
[a] Total number of larvae in the three replications.

Larvae were reared in three cohorts of 30 for a total of 90 insects per treatment. Individual neonates were added to 59-ml (2-oz) plastic rearing cups containing a cube of diet that had been weighed. The larvae were held in the growth chamber at a constant temperature of 29°C and a photoperiod of 16:8 (L:D) h until adult eclosion. Data on survival (percentage), diet consumption (milligrams), larval and pupal weight (milligrams), developmental time (days), adult deformity (percentage), adult longevity (days), fecundity (number of eggs), and adult dry weight (milligrams) were collected at 5, 10, and 15 d, and at pupation. Survival was calculated for five time intervals: neonate 5 d, 6–10 d, 11–15 d, 16 d to pupation, and neonate-to-adult eclosion. The pupation rate and the rate of adult eclosion from pupae also were recorded. The diet cubes were weighed after the fecal matter (loose material in the cup) was removed. The cups were cleaned with tissue paper to remove condensed moisture. Once pupation started, observations were made every 24 h to keep track of the remaining parameters. Percentage of pupation and percentage of adult emergence were calculated separately for males and females. Adults remained in the plastic cups until they died and then were frozen at −20°C and held for Rb and Cs analysis.

Mating of Adults Reared on RbCl, CsCl, and Control Diets. Male and female adults reared on the RbCl and CsCl diets were paired with individuals of the opposite sex from these two diets and from the control diet. Five pairings were made for each of the six mating combinations. Males and females from the control diet were not paired in this experiment because the numbers were too small. The adults were paired within 24 h of emergence and the pairs were held in 1.9-liter (2-qt) glass jars at 21 ± 2°C (room temperature). Wet cotton balls were provided to maintain humidity. Sheets of wax paper were provided for female oviposition. The number of eggs laid in each jar was recorded, and they were held at room temperature for neonate emergence to confirm mating and to obtain viability data. The dead adults were frozen at –20°C before processing for Rb or Cs analysis.

Detection of Rubidium and Cesium in Adults. *Graphite Furnace-Atomic Absorption Spectrophotometer (GF-AAS).* Adults were analyzed for Rb and Cs concentrations using the GF-AAS (model AA800, PerkinElmer Life and Analytical Sciences, Boston, MA) at Mississippi State University. The adults were oven-dried at 60°C for 4 d. Each adult was digested in 500 μl of nitric acid (70%) at 80°C for 1 h. Twenty microliters of this digested solution was added to 680 μl of nanopure water (35× dilution). Then, 30 μl was added to 270 μl of 2% nitric acid (total 350× dilution). Final concentration for injection onto the GF-AAS was 2%. Electrodeless discharge lamps were used. The samples were analyzed at wavelengths of 780 and 852.1 nm for Rb and Cs, respectively. Standard solutions of 1, 5, 10, and 20 ppb for Rb and 1, 2, 5, and 10 ppb for Cs were analyzed for calibration. The other conditions

Table 3. Larval and pupal weight (mean ± SEM) of male and female southwestern corn borer at three developmental stages when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Sex	n^a	Larval weight (mg)		Pupal weight (mg)
			10th day	15th day	
RbCl diet	Male	42	73.73 ± 7.65	172.47 ± 7.03bc	146.01 ± 10.25b
RbCl diet	Female	13	84.25 ± 7.65	257.53 ± 7.03a	210.78 ± 10.25a
CsCl diet	Male	31	71.72 ± 7.65	160.80 ± 7.03c	130.72 ± 10.25b
CsCl diet	Female	12	80.24 ± 7.65	234.10 ± 7.03a	178.80 ± 10.25ab
Control diet	Male	36	66.79 ± 7.65	194.67 ± 7.03b	155.27 ± 10.25ab
Control diet	Female	24	75.84 ± 7.65	257.90 ± 7.03a	213.54 ± 10.25a
F (df = 5, 12)			0.95	22.52	7.89
P			0.486	< 0.001	0.002

Means in columns followed by the same letter or no letter are not significantly different ($P > 0.05$, Student–Newman–Keuls, PROC GLM, LS MEANS).
[a] Total number of larvae or pupae in the three replications.

Table 4. Developmental time (mean ± SEM) of male and female southwestern corn borer over three time intervals when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Sex	n^a	Developmental time (d)		
			Larvae	Pupae	Neonate-adult eclosion
RbCl diet	Male	42	17.87 ± 0.38	8.87 ± 0.40	26.81 ± 0.51
RbCl diet	Female	13	18.47 ± 0.38	9.36 ± 0.40	27.80 ± 0.51
CsCl diet	Male	31	17.86 ± 0.38	9.27 ± 0.40	27.13 ± 0.51
CsCl diet	Female	12	18.31 ± 0.38	10.02 ± 0.40	28.33 ± 0.51
Control diet	Male	36	18.37 ± 0.38	9.70 ± 0.40	28.07 ± 0.51
Control diet	Female	24	18.95 ± 0.38	10.28 ± 0.40	29.23 ± 0.51
F (df = 5, 12)			0.75	1.58	3.05
P			0.599	0.238	0.053

Means in columns are not significantly different ($P > 0.05$ Student–Newman–Keuls, PROC GLM, LS MEANS).
[a] Total number of larvae or pupae in the three replications.

were set as specified for the AAS 800 (PerkinElmer 1995), except that the read time was set to 4 s and then 3 s.

Five male and five female adults reared on RbCl and CsCl diets were processed for GF-AAS for Rb and Cs concentration. The analysis for one female from CsCl diet malfunctioned and could not be used. Adults reared on control meridic diet were not processed, but the adults were analyzed for both Rb and Cs to get the background levels of Rb and Cs from adults reared on the other diet.

Neutron Activation Analysis (NAA). Adults were also processed for Rb and Cs concentrations by using the NAA at Kansas State University TRIGA MK-II (General Atomics, San Jose, CA) Nuclear Reactor Facility. No protocols existed for Rb or Cs detection by using NAA, and they 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) are maintained in a similar bell jar with desiccant in the laboratory. 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.

Each adult was weighed and packaged in 1.5 cc polyethylene sample vials. 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. The outer bag was labeled uniquely for each sample and standard by using a permanent marker. Packaged samples and standards were loaded in sets of six into polyethylene vials. Approximately 100 samples and standards were placed in the reactor for each irradiation; vials containing specimen 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 the outer bags covering each sample and the standard bag were removed and replaced with new (nonradioactive) bags to reduce chances of radioac-

Table 5. Statistical comparison of times to 50% pupation and 50% adult eclosion of southwestern corn borer when reared on RbCl- or CsCl-incorporated and control meridic diets at 29°C

	Comparison[a]	Developmental time (d)	Ratio (CL)[b]	Significance of[c]	
				Slope	Intercept
Pupation					
Males	RbCl vs. control	17.3 vs. 17.9	0.97 (0.93–1.00) NS	−	−
	CsCl vs. control	17.3 vs. 17.9	0.97 (0.93–1.01) NS	−	−
	RbCl vs. CsCl	17.3 vs. 17.3	1.00 (0.97–1.04) NS	−	−
Females	RbCl vs. control	17.8 vs. 18.8	0.95 (0.88–1.02) NS	−	−
	CsCl vs. control	17.8 vs. 18.8	0.95 (0.91–0.98)*	−	−
	RbCl vs. CsCl	17.8 vs. 17.8	1.00 (0.93–1.07) NS	+	+
Adult eclosion					
Males	RbCl vs. control	26.3 vs. 27.7	0.95 (0.93–0.97)*	−	+
	CsCl vs. control	26.5 vs. 27.7	0.96 (0.94–0.98)*	−	−
	CsCl vs. RbCl	26.5 vs. 26.3	1.01 (0.98–1.03) NS	−	−
Females	RbCl vs. control	27.5 vs. 28.7	0.96 (0.90–1.02) NS	−	−
	CsCl vs. control	26.8 vs. 28.7	0.93 (0.89–0.97)*	−	−
	RbCl vs. CsCl	27.5 vs. 26.8	1.03 (0.97–1.09) NS	−	−

NS, not significant.
[a] For each comparison, the numerator of the ratio is listed first.
[b] The two treatment developmental times differ when the confidence limits on their ratio does not include 1.
[c] +, Slope or intercept is significantly different at 0.05 level by using probit analysis of correlated data (Throne et al. 1995).

Table 6. Deformity (mean ± SE) and dry weight (mean ± SEM) of southwestern corn borer adults when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Sex	Adults eclosed[a]	Deformed adults (%)	n^a	Dry weight (mg)
RbCl diet	Male	42	17.84 ± 17.18	33	18.28 ± 2.95
RbCl diet	Female	13	11.11 ± 17.18	9	21.33 ± 2.95
CsCl diet	Male	31	39.49 ± 17.18	22	18.50 ± 2.95
CsCl diet	Female	12	44.44 ± 17.18	7	27.58 ± 3.61
Control diet	Male	36	26.19 ± 17.18	24	19.98 ± 2.95
Control diet	Female	24	29.05 ± 17.18	24	24.19 ± 2.95
F (df = 5, 12)			0.47		1.66
P			0.792		0.224

Means in columns are not significantly different ($P > 0.05$, Student–Newman–Keuls, PROC GLM, LS MEANS).
[a] Total number of adults in the three replications.

tive contamination. Sample information was transferred to the new bag. The samples were then held for 1 to 4 mo to allow the short half-life isotopes to decay and not interfere with Rb and Cs analysis. 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 was compared with the measured activity in the samples to determine concentration in the sample by using the Genie computer software (Genie 2000). The analysis of two male samples malfunctioned and could not be used. Rb or Cs concentrations in the adults detected with GF-AAS or NAA are expressed on dry matter basis as micrograms per gram (ppm).

Statistical Analysis. Data for the effects of RbCl and CsCl on various developmental parameters of the southwestern corn borer were analyzed as a nonparametric one-way analysis of variance (ANOVA) with ranked transformations (Conover 1999) by using PROC GLM (SAS Institute 1999–2000) that was based on evaluation of residuals for approximate normality by using stem and leaf plots. Differences in treatment means for diet consumption, larval and pupal weight, developmental time, adult deformity, adult longevity, fecundity of mated females, egg viability, and adult dry weight were analyzed for mean separation by Student–Newman–Keuls procedure (Sokal and Rohlf 1995). Differences in treatment means for survival and fecundity of virgin females were analyzed for mean separation by least significance difference (LSD) procedure (Sokal and Rohlf 1995). Student–Newman–Keuls was used in analysis when there were more than four treatment means, and LSD was used in analysis when there were less than four treatment means. Mean separations for the diet consumption, larval and pupal weight, developmental time, adult dry weight, adult longevity, and fecundity were weighted by sample size in each replicate for males and females. Data for developmental time to 50% pupation and adult eclosion were analyzed with probit analysis for correlated data by using log logit transformation for pupation and log probit transformations for adult eclosion (Throne et al. 1995). Data were checked for the homogeneity of slopes and intercept, and developmental times were compared across diet treatments (Robertson and Preisler 1992). A t-test was used to determine whether the proportion females differed from 0.5 in treatments. Data for the mated females in cross-mating studies were analyzed by chi-square for independence by using Fisher's exact test. For mated females, the number of eggs laid and the percentage of eggs viable were analyzed after square root transformations by using one-way ANOVA.

Table 7. Longevity and fecundity (mean ± SEM) of southwestern corn borer adults when reared on RbCl- or CsCl-incorporated and control meridic diets, and ANOVA results

Treatment	Sex	n^a	Longevity (d)	Eggs/virgin female
RbCl diet	Male	42	4.14 ± 0.14 c	
RbCl diet	Female	12	5.78 ± 0.14 a	43.83 ± 7.16
CsCl diet	Male	31	3.64 ± 0.14 d	
CsCl diet	Female	11	5.08 ± 0.14 b	28.09 ± 7.47
Control diet	Male	36	3.90 ± 0.14 c	
Control diet	Female	21	5.23 ± 0.14 b	38.81 ± 5.41
F (df)			27.01 (5, 12)	0.51 (2, 6)
P			<0.001	0.626

Means for longevity, Student–Newman–Keuls, or fecundity (LSD) followed by the same letter or no letter are not significantly different ($P > 0.05$, PROC GLM, LS MEANS).
[a] Total number of adults in the three replications.

Rubidium and Cs concentrations of adults also were analyzed as nonparametric one-way ANOVA by using PROC GLM that was based on evaluation of residuals for approximate normality by using stem and leaf plots. Differences in treatment means for Rb and Cs concentrations from GF-AAS or from NAA were analyzed for mean separation with the LSD procedure. To determine whether Rb and Cs concentrations changed over the male life span, the concentrations were also analyzed for males that lived 3 to 6 d as nonparametric one-way ANOVA by using PROC GLM. That was based on evaluation of residuals for approximate normality by using stem and leaf plots. Differences in treatment means were analyzed for separation by LSD procedure. Mean Rb or Cs concentrations from each replicate for each life span were weighted by sample size. The relationship between adult dry weight and Rb or Cs concentrations was analyzed using Spearman nonparametric correlation. All data have been back-transformed into the original units for presentation.

Table 8. Mated females, number of eggs per female (mean ± SE), and percentage of viability of the deposited eggs (mean ± SE) in various treatment combinations of males and females from RbCl- or CsCl-incorporated and control diets

Treatment		No. of pairs male:female	No. of mated females[a]	Avg. no. eggs/female[b]	Egg viability (%)[b]
Rb-marked male	Unmarked female	5	0	0	
Unmarked male	Rb-marked female	5	1	29.00 ± 35.85	10.34 ± 24.20
Cs-marked male	Unmarked female	5	2	40.50 ± 25.35	32.24 ± 17.11
Unmarked male	Cs-marked female	5	1	26.00 ± 35.85	15.34 ± 24.20
Rb-marked male	Cs-marked female	4	2	101.50 ± 25.35	7.36 ± 17.11
Cs-marked male	Rb-marked female	5	2	67.00 ± 25.35	7.55 ± 17.11

[a] Mated females are not significantly different across treatments (chi-square test, df = 5, $P = 0.659$).
[b] Means in columns are not significantly different for eggs per female ($F = 3.62$; df = 4, 3; $P = 0.160$) and egg viability ($F = 0.69$; df = 4, 3; $P = 0.644$), Student–Newman–Keuls, PROC GLM, LS MEANS).

Results

Effects of RbCl and CsCl on Southwestern Corn Borer. The main effects of treatments on survival (percentage) during the different developmental periods were not significant (Table 1). Survival also did not differ across treatments when analyzed as pupation rate, neonate-to-adult eclosion, and adult eclosion from pupae (Table 1). The main effects of treatments on diet consumption (milligrams) by larvae during the different developmental periods were not significant (Table 2). Larval weights (milligrams) of neonate-to-5-d-old larvae were not large enough to be measured with precision. The main effects of treatments on larval weight at 10 d of larval development were not significant (Table 3). At 15 d of larval development, male and female larval weights were different across treatments (Table 3). Females were heavier than males within each treatment (Table 3). Female weights were not different across treatments; however, control males were heavier than males reared from CsCl diet (Table 3). The main effects of treatments on weight (milligrams) of male and female pupae were significant (Table 3). Females were heavier than males on the RbCl diet (Table 3). Male or female weights did not differ across treatments (Table 3). Females from control and RbCl diets were heavier than males from RbCl and CsCl diets (Table 3).

The main effects of treatments on developmental time (days) of male and female larvae and pupae were not significant (Table 4). Male pupation started 16 d after inoculation for all diets. Female pupation started 16 d after inoculation for the control diet and after 17 d for the RbCl and CsCl diets. For males, the mean developmental time to 50% pupation did not differ across diets, but females developed faster on CsCl diet than did females on the control diet (Table 5). Slope and intercept were significant for one of the three comparisons across diets for females, but not for males (Table 5). Male eclosion started 25 d after inoculation for the RbCl diet and after 26 d for the control and CsCl diets. Female eclosion started 26 d after inoculation for the RbCl and CsCl diets and after 27 d for the control diet. Mean developmental time to 50% adult eclosion was lower for RbCl and CsCl diets than for control diet for males, and it was lower for the CsCl diet than for the control diet for females (Table 5). Proportion female in the control (0.38 ± 0.12) and CsCl (0.24 ± 0.09) diets did not differ from the expected proportion of 0.5 ($t = -0.90, -3.03$; df = 2, 2; $P = 0.461, 0.094$), but proportion female from the RbCl (0.23 ± 0.02) diet was lower than the expected proportion of 0.5 ($t = -13.28$, df = 2, $P = 0.005$).

The main effects of the treatments on adult deformity and dry weight were not significant (Table 6). Longevity (days) of male and female adults was dif-

Table 9. Rubidium and cesium concentration (mean ± SE) of male and female southwestern corn borer adults when reared on RbCl- or CsCl- incorporated and control diets, by using the GF-AAS

Sex	Diet	*n*	Mean ± SE	Range	Limits = Mean + 3 SD	No. exceeding Rb or Cs limits
Rb content (ppm)[a]						
Males	RbCl	5	487.38 ± 73.91a	193.01–804.48		5
	CsCl	5	2.50 ± 73.91b	0.81–4.74	7.10	0
Females	RbCl	5	1154.48 ± 58.51a	942.52–1410.20		5
	CsCl	4	2.66 ± 65.48b	1.21–4.01	6.62	0
Cs content (ppm)[b]						
Males	RbCl	5	1.75 ± 24.10a	0.08–5.11	7.98	0
	CsCl	5	98.71 ± 24.10b	9.35–207.77		5
Females	RbCl	5	3.36 ± 91.05a	2.12–6.46	8.74	0
	CsCl	4	359.10 ± 101.80b	12.65–725.20		4

Means in columns followed by the same letter for males and females, within Rb or Cs sections, are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).
[a] ANOVA RbCl males: $F = 25.0$; df = 1, 8; $P = 0.001$; and females: $F = 21.0$; df = 1, 7; $P = 0.003$.
[b] ANOVA CsCl males: $F = 25.0$, df = 1, 8; $P = 0.001$; and females: $F = 21.0$; df = 1, 7; $P = 0.003$.

Table 10. Rubidium and cesium concentration (mean ± SE) of male and female southwestern corn borer adults when reared on RbCl- or CsCl- incorporated and control diets, by using the NAA

Sex	Diet	*n*	Mean ± SE	Range	Limits = Mean + 3 SD	No. exceeding Rb or Cs control diet limits
Rb Content (ppm)[a]						
Males	Control	24	0.00a			0
	RbCl	33	904.37 ± 78.96b	126.30–3089.61		33
	CsCl	20	0.00a			0
Females	Control	24	0.00a			0
	RbCl	9	826.80 ± 96.13b	304.00–1672.51		9
	CsCl	7	0.00a			0
Cs content (ppm)[b]						
Males	Control	24	0.47 ± 55.87a	0.00–4.39	6.03	0
	CsCl	20	503.72 ± 61.21b	10.40–1597.10		20
	RbCl	33	2.60 ± 47.65c	0.00–11.17	11.97	4
Females	Control	24	0.67 ± 38.20a	0.00–3.57	4.50	0
	CsCl	7	546.22 ± 70.71b	103.34–1222.63		7
	RbCl	9	1.69 ± 62.36a	0.00–5.90	6.10	2

Means in columns followed by the same letter for males and females, within Rb or Cs sections, are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).
[a] ANOVA RbCl males: $F = 345.7$; df = 2, 74; $P = 0.001$; and females: $F = 860.3$; df = 2, 37; $P = 0.001$.
[b] ANOVA CsCl males: $F = 83.2$; df = 2, 74; $P = 0.001$; and females: $F = 20.4$; df = 2, 37; $P = 0.001$.

ferent across treatments (Table 7). Females lived longer than males within each treatment. Males from the RbCl and control diets lived longer than males from the CsCl diet. Females from the RbCl diet lived longer than females from CsCl and control diets. The main effects of the treatments on the number of eggs laid by virgin females were not significant (Table 7).

The main effects of the treatments on mating success of males and females were not significant according to the chi-square analysis (Table 8). There were no more than two mated females in any of the treatments (out of five pairs), and no mated female was present in the treatment consisting of males from RbCl diet and females from control diet. Females were considered mated if neonates hatched from the deposited eggs. There were no differences across treatments in the number of eggs deposited by mated females or in the percentage of eggs that were viable (Table 8).

Rubidium and Cesium Concentrations in Adults. *GF-AAS.* Rubidium concentrations of males and females were higher for adults reared on RbCl diet than for adults reared on CsCl diet (Table 9). Conversely, both male and female Cs concentrations were higher for adults reared on CsCl diet than for adults reared on RbCl diet (Table 9). A small amount of Rb was detected in adults reared on CsCl diet, and a small amount of Cs was detected in adults reared on RbCl diet (Table 9). If a threshold for detection is set at background mean ± 3 SD (in this case, Rb or Cs in the opposite treatment), then 100% of the adults from either of the treated diets could be considered marked (Table 9).

NAA. In total, 119 adults were available for Rb or Cs analysis with NAA, including 33 males and nine females from RbCl diet, 22 males and seven females from CsCl diet, and 24 males and 24 females from the control diet. The Rb and Cs concentrations of males and females from RbCl and CsCl diets were higher than for respective adults from the control diet (Table 10). The Rb concentrations of adults from the control diet did not reach the detection limit for NAA and are recorded as zero. Rubidium also was not detected in the adults from CsCl diet (Table 10). Because all adults from the RbCl diet had detectable amounts of Rb, they could all be effectively distinguished from adults from the control and CsCl diets and were rated as being marked. Even at a threshold of mean ± 3 SD or the maximum Rb concentration detected using AAS in the adults from CsCl diet, 100% of male and female adults from RbCl diet were marked with Rb. Detectable amounts of Cs were measured in 29.17% of males and 29.17% of females from the control diet (Table 10). Detectable amounts of Cs were also measured in 66.67% of males and 33.33% of females reared on RbCl diet (Table 10). Using a threshold of mean ± 3 SD for Cs in adults from control diet or in adults from RbCl diet, 100% of the male and female adults from CsCl diet were marked with Cs. The Cs detection in adults from RbCl diet was higher compared with Cs in the adults from the control diet. There were no differences in Rb or Cs concentrations for males of different ages (Table 11). There was considerable variation in the Rb or Cs concentrations of males of the same age. Females of

Table 11. Rubidium and cesium concentration (mean ± SEM) of male southwestern corn borer that died at different days, and ANOVA results

Day	RbCl diet		CsCl diet	
	n[a]	Rb (ppm)	*n*[a]	Cs (ppm)
3	9	1031.1 ± 205.52	10	676.6 ± 205.52
4	13	839.9 ± 205.52	8	431.1 ± 251.71
5	9	818.1 ± 205.52	2	254.9 ± 251.71
6	2	1137.6 ± 251.71		
F (df)		0.94 (3, 7)		3.01 (2, 4)
P		0.472		0.159

Means in columns are not significantly different ($P > 0.05$, LSD, PROC GLM, LS MEANS).
[a] Number of males from three replications.

different ages were not compared for Rb or Cs concentrations.

Rubidium and Cs were not detected in control males that were paired with females reared on RbCl or CsCl diets. Rb and Cs were also not detected in most control females that were paired with males reared on RbCl or CsCl diets, but there was one female that had detectable levels of Cs (1.56 μg/g). There was a positive correlation between adult dry weight and Rb ($r = 0.42$, df = 41, $P [r > 0] = 0.006$) or Cs ($r = 0.58$, df = 26, $P [r > 0] = 0.002$) concentrations (detected by NAA).

Discussion

Most fitness indicators such as survival, diet consumption, larval and pupal weight, developmental time, adult deformity, adult dry weight, and fecundity of the virgin and mated females were not different across RbCl, CsCl, and control diets. Other researchers have reported few negative effects of Rb and Cs for other insects on these development parameters when RbCl (or Rb) and CsCl (or Cs) were used at rates of ≈1000 μg/g in the artificial diet: tobacco budworm, *Helicoverpa virescens* (F.) (Graham and Wolfenbarger 1977); *Trichoplusia ni* (Hübner) (Stimmann et al. 1973, Stimmann 1974); pink bollworm, *Pectinophora gossypiella* (Saunders); (*Trichoplusia ni* (Hübner) (Moss and Van Steenwyk 1982, 1984), European corn borer (Legg and Chiang 1984); tufted apple bud moth, *Platynota idaeusalis* (Walker) (Knight et al. 1989); and gypsy moth adult, *Lymantria dispar* (L.) (Johnson and Reeves 1995). Van Steenwyk et al. (1978) found slight effects on larval development of the pink bollworm at 8,550 μg/g Rb in the diet. Stimmann et al. (1973) found slower larval development of cabbage looper on a diet with Rb concentrations of up to 70,000 μg/g; however, the rate of pupal development was unaffected. Holbrook et al. (1991) found that 500 and 1000 μg/g Rb in the rearing media of the biting midge *Culicoides variipennis* (Coquillett) reduced pupal production, adult emergence, and adult longevity. In our study, there seemed to be only minor effects associated with the use of RbCl and CsCl at 1000 μg/g in the meridic diet. There was a difference in weight in the 15 d of larval development between treatments, but this was mainly associated with differences across sex. Males reared on CsCl diet had lower weight than males from RbCl or control diets; however, the differences were not significant in male or female pupal weights, and the sex-linked difference was significant only in adults from RbCl diet. Weight differences between male and female pupae and adults have been reported previously for the southwestern corn borer (Davis 1965, Ng et al. 1993). Although developmental time to 50% pupation or adult eclosion was significant across diets for some comparisons, the differences ranged only from 1 to 1.5 d. Sex ratio was affected in the RbCl diet. There was considerable variation in the number of females recovered in different replicates, particularly in control and CsCl diets. Such variations could be random or due to unequal distribution of the sexes across treatments at the time of inoculation.

Food consumption and utilization by an insect may vary with age and sex and with the amount, rate, and quality of the diet. These parameters influence insect growth rate, developmental time, and final body weight (Waldbauer 1968, Slansky and Scriber 1985). There were no differences in the diet consumed by males or females across treatments, but female larvae consumed more diet than male larvae in all the age groups. In part, this explains the weight difference between sexes on the 15 d of development and in pupal weight. This confirms the observations reported by Ng et al. (1993) that diet consumption was associated with size dimorphism of southwestern corn borer larvae and pupae. They also reported the occurrence of great variation in the larval weight of southwestern corn borers even within the same age group when reared on the same maize genotypes or the same meridic diet. Longevity of adults was different for males and females across treatments; however, the differences were no more than a day. Rubidium chloride seemed to increase male and female longevity, and CsCl seemed to decrease male longevity compared with adults from the control diet. Ng et al. (1993) reported a life span of 3–5 d for southwestern corn borer adults, which was similar to that reported in the current study. Only a few individuals lived for as long as 6 d. Knight et al. (1989) found no effects on the fecundity or longevity of the tufted apple bud moth reared with 3,000 μg/g Rb; however, at 6000 μg/g Rb the females died earlier, deposited fewer egg masses, and produced fewer eggs than females reared on control diet.

Male eclosion from pupae reared on RbCl and control diets started 1 d earlier than did female eclosion. This allows males to emerge before females to improve their competitive advantage in mating. The emergence of males and females was 2–3 d earlier for pupae reared on RbCl and CsCl diets than for those reared on the control diet. This suggests that the treatments may accelerate development. A similar trend was observed for time to pupation.

The frequency of wing deformity among the adults did not seem to be associated with the RbCl or CsCl treatments, because the numbers of deformed adults were similar across treatments. Stimmann et al. (1973) reported that the incidence of deformed adults in cabbage looper increased only when Rb concentration reached 28,000 μg/g. The frequency of wing deformity among the adults is a concern relative to the viability of laboratory-reared insects. In a subsequent study, we changed the antibiotic package in an effort to improve the colony performance, but the frequency of wing deformities remained between 17 and 27% (Qureshi et al. 2004). Wing deformity seems to be a problem with southwestern corn borer diet or rearing conditions. Reddy and Chippendale (1972) reported that several nutrient deficiencies were associated with wing deformities. The insects also may have more problems emerging and expanding the wings in the rearing cups than they would have in tunnels in plants.

In this study, mating success was <40% across treatments, probably because most southwestern corn borers mate during the night of emergence (Rolston 1955). In this study, the moths were paired for mating after the first night. The short life span (3–5 d) and lack of synchrony in emergence makes it less likely that moths in the sexually active stage will be available for mating at the same time in small mating arenas. In this study, mating did not occur between males from RbCl diet and females from control diet. However, mating between males and females from the other five mating combinations indicates that males and females do mate regardless of the diet on which they were reared. Males and females from the control diet were not paired in this experiment, but they are known to mate successfully and yield 65% mated females (sex ratio was 2:1 male to female and paired as pupae) (Qureshi et al. 2004).

Viability of eggs (based on hatching of neonates) was low in these tests. This probably is also an indication of low mating success (rather than effects of RbCl and CsCl treatments). Virgin southwestern corn borer females commonly lay unfertilized eggs. Many of the eggs may have been laid before the females mated. Low egg viability was reported in the soybean looper, *Pseudoplusia includens* (Walker), where adults were fed artificial nectar containing 190 μg/g RbCl or CsCl (Jost and Pitre 2002). However, most other lepidopterans treated with >1000 μg/g Rb or Cs mate successfully (Stimmann et al. 1973; Graham and Wolfenbarger 1977; Moss and Van Steenwyk 1982, 1984; Hayes and Reed 1989).

GF-AAS and NAA were both effective in detecting Rb and Cs in adults that resulted from larvae reared on RbCl and CsCl diets. There were differences in Rb and Cs concentrations among adults reared on RbCl, CsCl, or control diets. Males and females from RbCl and CsCl diets contained enough Rb or Cs to differentiate them from adults reared on the control diet. Marker level in the marked adults was higher than the mean $\pm$ 3 SD or the maximum marker level detected in the control adults.

Mean Rb and Cs concentrations for females reared on RbCl and CsCl diets were double those for males when analyzed using AAS, suggesting that females likely accumulate more Rb or Cs in their bodies than do males. Minimum Rb concentration detected in the females was higher than the maximum Rb concentration detected in the males. Three of the four females had higher Cs concentrations than the maximum Cs concentration in males. Cesium concentration for one male and one female from CsCl diet was surprisingly low compared with Cs concentrations of other males and females from the same diet. No such cases were observed for Rb concentrations in males and females from RbCl diet. Higher levels of Rb have been reported in females in other studies (Stimmann et al. 1973, Graham and Wolfenbarger 1977, Van Steenwyk et al. 1978, Legg and Chiang 1984). Moss and Van Steenwyk (1982, 1984) found no difference in the Cs concentrations of the male and female adults of the pink bollworm and cabbage looper reared on diets containing 10^{-2} and 10^{-3} M CsCl. Sexually dimorphic uptake of Rb may represent the additional biomass incorporation into the ovaries and eggs of the females (Stimmann et al. 1973, Knight et al. 1989). However, in our studies almost all the females processed for Rb or Cs concentration had oviposited. In the GF-AAS analysis, Rb was detected in all CsCl diet adults and vice versa, but the Rb and Cs levels were much lower than for adults from RbCl and CsCl diets.

Mean Cs concentration was higher in females than in males reared on CsCl diet; however, the mean Rb concentration was higher in males than in females reared on RbCl diet by using the NAA. Differences in the mean Rb and Cs concentrations for males and females were not very large. Background levels of Rb in the adults from control and CsCl diets were not high enough to be detected using NAA; however, Cs was detected in males and females reared on RbCl diet. Rubidium and Cs detected in adults from RbCl and CsCl diets were variable. Rubidium concentration was 100–500 μg/g in 30.3%, 500-1000 μg/g in 33.3%, and >1000 μg/g in 36.4% of males from RbCl diet. Rubidium concentration was 100–500 μg/g in 55.5%, 500-1000 μg/g in 11.1%, and >1000 μg/g in 33.3% of females from RbCl diet. Cesium concentration was <100 μg/g in 25%, 100–500 μg/g in 35%, 500-1000 μg/g in 20%, and >1000 μg/g in 20% of males from CsCl diet. Cesium concentration was <100 μg/g in 14.3%, 100–500 μg/g in 42.9%, 500-1000 μg/g in 14.3%, and >1000 μg/g in 14.3% of females from CsCl diet.

The time interval between the removal of insects from the marked diet and their death has been reported to be an important factor in the decline of marker over time in some studies but not in others. Legg and Chiang (1984) found no significant change in Rb concentration over the adult life span of European corn borers reared on RbCl diets (10,000 μg/g Rb). Moss and Van Steenwyk (1982, 1984) found that Cs marker was sufficient to last for the expected life of the cabbage looper and the pink bollworm reared on artificial diets (10^{-2} M CsCl). Culin and Alverson (1986) found that adult male *Helicoverpa zea* (Boddie) fed on artificial nectar spiked with 5,000, 10,000, or 20,000 μg/g RbCl retained significant marker for up to 12 d postfeeding. Holbrook et al. (1991) found that adult females of the biting midge reared on media containing 15.6 μg/g Rb were marked for a period of at least 13 d. Cohen and Jackson (1989) found that 28% of the average Rb concentration remained after 7 d, but only 7.4% of the original Rb concentration remained after 14 d in *Geocoris punctipes* (Say). Frazer and Raworth (1974) found that in adult pea aphids, *Acyrthosiphon pisum* (Harris), 77% of Rb was lost within 2 d of leaving the source plant but that detectable quantities were present for 4 d. Guillebeau et al. (1993) reported that the level of Rb in green peach aphid, *Myzus persicae* (Sulzer), and potato aphid, *Macrosiphum euphorbiae* (Thomas), was reduced by nearly 90% after a 5-d removal from the tomato plants treated with 10,000 μg/g RbCl. Fleischer et al. (1986) found that once removed from the Rb-enriched host material, Rb concentration in adult *Lygus lineolaris*

(Palisot de Beauvois) declined exponentially. In the current study, there were no statistically significant differences in the Rb or Cs concentrations of the male adults that lived 2 and 6 d. There was considerable variation in the Rb or Cs concentrations of adults that lived the same number of days. The durability of marked adults is not important for southwestern corn borer because they live only for 3–5 d and do not feed as adults. There should be little decay of Rb and Cs concentrations. The absence of feeding also reduces the risk of marker loss through excretion (Chapman 1982, Jost and Pitre 2002).

There was a positive correlation between the dry weight of the adults and their Rb or Cs concentrations. This correlation was slightly better for Cs concentrations than for Rb concentrations. Insects that consumed more diet seemed to accumulate more of the marker. Also, the NAA depends on the mass of an element in a sample for detection. Sometimes single insects did not have enough mass of the element to be detected, even when a previous multiple-insect sample indicated a marked individual was present. There seemed to be a lower detection rate in lower body weight insects. The results presented here were only from the individually processed moths.

Rb or Cs were not detected in females paired with males marked with the other element, except in one case where a small amount of Cs was detected in a female that was paired with a Cs-marked male. The marker concentration may not be high enough in males to transfer enough marker to be detected by the NAA. Improved instrumentation with lower detection limits may be required to detect Rb and Cs transferred by mating. Horizontal (mating) and vertical (oviposition) transfer of Rb and Cs has been reported for several lepidopterans: tobacco budworm (Graham and Wolfenbarger 1977, Hayes and Reed 1989), pink bollworm (Van Steenwyk et al. 1978), cabbage looper (Moss and Van Steenwyk 1984), and soybean looper (Jost and Pitre 2002). However, 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 European skipper contained 2–3 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 because the average egg complement contained >50% of the total body sodium of females.

Several factors could contribute to the Rb concentration being higher than Cs concentration for both males and females. The atomic weight of Cs is higher than that of Rb, so there will be more molecules of Rb than Cs in the diet for the same gravimetric weight of RbCl and CsCl. The insects may also be more efficient in accumulating Rb than Cs, although both elements mimic potassium in biological systems (Jost and Pitre 2002). It is also possible that the two detection instruments (GF-AAS and NAA) have different efficiencies in detecting Rb and Cs.

This study has shown that either RbCl or CsCl at the rate of 1000 μg/g in the meridic diet exert only minor effects on the development or behavior of southwestern corn borer and yield adults that were successfully marked with Rb or Cs. Therefore, RbCl and CsCl can be used to mark southwestern corn borer adults with Rb and Cs for use in dispersal experiments.

Rubidium chloride and CsCl also can be sprayed on maize foliage at 1000 ppm to mark southwestern corn borers developing in the plants (Qureshi 2003). Whenever feasible, it would be preferable to use insects reared on RbCl- and CsCl-marked maize plants. They would be better representatives of feral insects and would provide a more realistic picture of the dispersal biology of the insect. However, it is difficult to manage production of insects in field grown plants. First generation corn borers would have to be reared the previous year and survive the winter in plant stubble to emerge the following year to participate in the first flight. Field-grown insects also emerge at the same time as the feral populations emerge. This is good timing for some studies, but it also means that large numbers of trapped insects will need to be processed to identify marked insects. Laboratory-reared insects can be released at other times to study, for example, the influence of changes in crop phenology or canopy or weather conditions on dispersal behavior. They also can be released at nonflight times simply to reduce the number of captured feral insects that have to be processed. There will be a continuing need for marked laboratory-reared insects for certain experimental situations.

Elevated levels of Rb and Cs in southwestern corn borer adults can be successfully detected using the GF-AAS or the NAA. GF-AAS seemed to be more sensitive than NAA, but NAA may have enough sensitivity to detect marked adults for dispersal studies. We made special arrangements to do these preliminary experiments, so a true cost comparison of the two techniques was not available. Therefore, the choice of which technique to use in future studies will depend on the local availability, local cost, and/or local convenience for each technique. The specific objective of the study also may be important in the selection of technique.

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