Environmental Entomology, 45(2), 2016, 427–435
doi: 10.1093/ee/nvw009
Advance Access Publication Date: 26 February 2016
Research article

Labeling Feral Spruce Budworm (Lepidoptera: Tortricidae) Populations With Rubidium

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Received 15 September 2015; Accepted 29 January 2016

Abstract

Rubidium (Rb) is a trace element that occurs naturally in low concentrations and is easily absorbed by plants, making it a useful tool for labeling insect defoliators, such as spruce budworm (*Choristoneura fumiferana* Clemens). Balsam fir trees (*Abies balsamea* (L.) Miller) injected with either 8 or 16 g per tree of rubidium chloride (RbCl) showed quick uptake and distribution throughout the crown, with no negative effects on tree shoot growth or spruce budworm survival and development. Adult spruce budworm that fed as larvae on trees injected with RbCl were clearly labeled, with significantly higher Rb concentrations than the background levels found in adults that fed as larvae on control trees. Rb concentrations in feral spruce budworm adults for both the 8 g (9 μg/g) and 16 g (25 μg/g) per tree treatments were at least five times lower than those in laboratory-reared adults on 1,000 μg/g RbCl diet (125 μg/g); survival, development, pupal weight, sex ratio, and mating status of spruce budworm were not adversely affected by Rb treatment. Egg masses laid by feral females that fed as larvae on Rb-labeled trees were also labeled with Rb. Injecting trees with RbCl is a viable technique for labeling feral spruce budworm populations to help distinguish local populations from immigrants to better evaluate the success of early intervention strategies such as mating disruption.

Key words: insect labeling, rubidium chloride, spruce budworm, systemic tree injection

Knowledge of the movement of insects in their natural environment is essential for a better understanding of the processes underlying their population ecology, and a number of techniques have been developed to mark and track the movement of individuals. For these techniques to be useful, they should not alter the insect's behavior and survival, and the marker needs to persist long enough and at high enough concentrations in or on the insects for it to be measured (Southwood 1978). Of all the techniques used to label insects, the most promising is the use of trace elements of alkali metals, such as rubidium (Rb) and cesium (Cs) (Hopper 1991). The use of the chloride form of Rb to label insects was first proposed by Berry et al. (1972) for the following reasons: Rb can be found in low concentrations almost everywhere; it is nonradioactive and, therefore, does not pose a risk to the environment; it is nontoxic to insects; it is relatively easy to administer and highly water soluble; and it is relatively simple to detect and determine low concentrations in animal and plant tissues. Rb is also easily absorbed by plants and can both move through the phloem and be ingested by herbivores; thus, it is very useful for labeling herbivores (Jackson 1991). The chemical properties of Rb are very close to those of potassium (K), and it can replace or partially replace K in living organisms (Stimmann et al. 1973).

The spruce budworm is a forest insect defoliator that feeds on the current foliage of spruce and balsam fir trees and can kill its host tree species through repeated annual removal of the current foliage. Historically, spruce budworm outbreaks tend to occur about every 30 yr (Royama 1984), causing severe widespread tree mortality across the landscape. In New Brunswick (NB), Canada, spruce budworm populations have been low for the past 25 to 30 yr and are currently beginning to increase. A proactive approach called "early intervention strategy" is being implemented to try to keep populations low to mitigate the severe widespread damage that is typically caused by these periodic outbreaks. One strategy being evaluated is to apply biological insecticides and spruce budworm pheromones to areas with rising populations in an attempt to keep populations below where extensive damage occurs on the trees. However, migration of moths from outside the treated areas can have a confounding influence on posttreatment population assessments, making it difficult to evaluate the success of these early intervention trials. Labeling local spruce budworm populations could be a very useful technique to help distinguish local spruce budworm from immigrants in treatment areas, enabling a more accurate evaluation of the success of these trials.

In this study, we injected balsam fir trees with an aqueous solution of RbCl (either 8 or 16 g per tree) to evaluate its potential as a marker in feral adult spruce budworm that fed as larvae on the current-year foliage of these trees. As complementary work to this

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study, we reared spruce budworm larvae on RbCl-incorporated artificial diet to quantify the uptake of Rb by spruce budworm and to determine the effects on spruce budworm survival and development rate.

Materials and Methods

Laboratory Diet Trial

To determine if Rb negatively affects the survival, development, size, sex ratio, and mating success of spruce budworm, second-instar (L_2) spruce budworm obtained from the Insect Production Unit of the Canadian Forest Service, Sault Ste. Marie, ON, Canada, were reared on three different diets: standard McMorran artificial diet (McMorran 1965); standard McMorran diet with RbCl (99% pure, Sigma Aldrich, St. Louis, MO) added at two concentrations—1,000 µg/g (1 g/liter of wet diet) and 10,000 µg/g (10 g/liter of wet diet). The 1,000 µg/g concentration has been proven to mark the southwestern corn borer (*Diatraea grandiosella* Dyar) with no significant effects on insect health (Qureshi et al. 2004a). The 10,000 µg/g concentration was used to test the spruce budworm's tolerance to relatively high concentrations of Rb. In all treatments, diet was poured into trays to a depth of ~1 cm; plugs of diet were removed by cutting the diet using the open end of a glass vial (17 by 60 mm^2; 2 dram); and the plug was then pushed to the bottom of the vial. The L_2 larvae were randomly selected from the colony, with one larvae placed into each vial, for a total of 140 larvae for each of the three treatments. The vials were sealed using foam plugs and stored upside down with the diet upward. The vials were monitored daily to track mortality and the development time to reach sixth instar (L_6), pupal stage, and adult eclosion. The experiment was conducted in a laboratory at a constant temperature of 22°C, ~40% relative humidity, and a photoperiod of 16:8 (L:D) h.

Adults from the three treatments were used to compare mating success among treatments. For each treatment, up to ten 2- to 3-d-old males and ten 1- to 2-d-old females per replicate were placed in mesh cages (53 cm long by 25 cm diameter). The control and 1,000 µg/g treatments were each replicated six times (i.e., six cages per treatment; $n = 6$) and the 10,000 µg/g treatment five times ($n = 5$). All replicates (cages) for each treatment were placed in separate rooms at ~22°C under a photoperiod of 16:8 (L:D) h. The pairs were left to mate for 24 h, and the mating status of the females was determined by removing the bursa copulatrix and checking under a dissecting microscope for the presence or absence of a spermatophore.

Site Selection for Field Experiment

The study area for the Rb experiment was located in the Gaspé Region of Quebec just north of the town of Amqui (latitude 48.583933, longitude −67.517218). The study area was selected based on the following criteria: moderate to severely defoliated by spruce budworm in 2013; high populations forecast in the area for 2014 based on overwintering L_2 counts; located outside the planned areas targeted for aerial application of insecticides in 2014; contained stands predominantly consisting of mature balsam fir; and the area is a parcel of Quebec Provincial Crown land. Balsam fir stands were selected to establish a 50- by 120-m treatment block for each of the three treatments used in this study; Block 1 for control, Block 2 for the 8 g per tree RbCl treatment, and Block 3 for the 16 g per tree RbCl treatment. Treatment blocks were located at least 50 m from the nearest road, with a minimum distance of 150 m between each block. A research permit was obtained from the Quebec Ministry of Natural Resources to conduct field trials on these parcels of land.

Stand Characteristics and Spruce Budworm Populations

To quantify the stand structural characteristics, an 11.3-m (0.04 ha) fixed-radius plot was established in the center of each of the three blocks. Species, diameter at breast height (dbh [1.3m]), crown class, tree status (dead or alive), crown health, and damage were recorded for each tree within the plot. Increment cores and tree heights were taken from five dominant or codominant balsam fir trees within the fixed-radius plots.

The three blocks consisted primarily of balsam fir, ranging from 60 to 86% of the stand basal area, with other softwoods and hardwoods as minor stand components (Table 1). Tree height ranged from 18.7 to 21.0 m, and the average stand age was 48, 42, and 60 yr for control, 8 g, and 16 g per tree, respectively (Table 1).

To quantify spruce budworm population densities within each block, a 75-cm midcrown branch sample was taken from five randomly selected balsam fir trees, and an L_2 wash and count was performed on the branches (Hartling 1994). As shown in Table 1, populations ranged from 6,235 to 15,691 larvae per 10 m^2, which are considered to be high spruce budworm population densities (Dorais and Kettela 1982). This confirmed that the blocks selected were suitable for this field experiment because each contained a significant abundance of spruce budworm to sample and analyze for larval development and Rb concentrations.

Sample Tree Selection

Within each of the three blocks, 10 balsam fir trees between 18 and 26 cm dbh were randomly selected as sample trees. Sample trees were spaced at least 20 m apart and extended the entire length of the block. Each tree was sequentially numbered (control = 1 to 10; 8 g per tree treatment = 11 to 20; and 16 g per tree treatment = 21 to 30) and painted with yellow spray paint to keep track of subsequent samples taken from each tree and associated treatments. All vegetation around each sample tree was cleared to provide access for pole pruning. Any branches overlapping the crown were removed to minimize the movement of larvae crawling from a neighboring tree onto the sample tree.

Sample trees from the treated blocks were injected with RbCl solution using the Ecoject Micro Injection System developed by BioForest Technologies Inc. (Sault Ste. Marie, ON, Canada). Four holes 6 mm in diameter were drilled at a 45 degree angle ~30 cm from the base of the tree in four cardinal directions (N, S, E, W). Another four holes of the same size, and at the same angle, were drilled ~75 cm from the base in four directions (NE, SE, SW, and NW) above the holes drilled at 30 cm from the base. Nozzles were inserted into the holes, and canisters filled with RbCl solution were connected to the nozzles. Each canister contained either 1 mg or 2 mg of RbCl for the 8 and 16 g per tree treatments, respectively. The pressure inside the canister forced the RbCl solution into the phloem of the tree. Once the canisters were emptied of RbCl solution, the nozzles were removed, and the holes were filled with tree wax. All sample trees were injected on 27 May 2014, the time of bud burst of balsam fir in the study area. This timing for injection maximizes the probability of Rb being incorporated into the tissue of new shoots because the vascular system of the trees is actively supplying water and nutrients to support new shoot growth at that time. Preliminary research (P. S., unpublished data) indicated that it may take ~3 wk for maximum Rb concentrations to get into the

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Table 1. Species composition, average height and age, and L_2 counts for the three blocks near Amqui, QC

Block	Treatment	Species basal area (%)				Tree height (m)	Tree age (yr)	L_2 count/10 m^2
		BF	SP	OS	HW			
1	Control	60	16	16	7	20.8	48	6,235
2	8 g per tree	74	0	17	9	18.7	42	10,225
3	16 g per tree	86	6	1	8	21.0	60	15,691

BF, balsam fir; SP, spruce; OS, other softwood; and HW, hardwood.

new shoots throughout the crown. This coincides with the time that spruce budworm reach fifth instar (L_5) and L_6, when >87% of the spruce budworm's total consumption of current-year foliage is eaten (Miller 1977).

Branch Sampling

Pole pruners were used to take midcrown branch samples (75 cm) from each of the 30 sample trees once a week starting from 2 June to 17 July with collections gathered once daily between 15 and 17 July for egg masses. All branch samples were placed in brown paper bags and labeled with tree number and collection date. All samples were placed in a cooler with ice packs to maintain spruce budworm developmental condition and to slow their movement. Branch samples were transported back to the Canadian Forest Service (CFS) lab in Fredericton, NB, Canada, for processing. In total, 150 larvae (50 randomly selected from each of the three treatments) were collected from branches on 24 June (the start of the pupal stage) and used to measure head capsule widths for spruce budworm development. The pupae found on each branch were sexed (male or female), weighed (to the nearest mg), and placed in rearing, with daily checks for adult emergence. Adults and egg masses were also extracted from the branches and frozen at −20°C for subsequent Rb analysis.

Growth Measurements

To avoid the confounding influence of heavy defoliation in 2013 and ongoing defoliation in 2014 on shoot growth and development in Amqui, nine healthy balsam fir trees between 18 and 26 cm dbh were selected in the University of New Brunswick (UNB) woodlot in Fredericton, NB (latitude 45.924075, longitude −66.650848) to quantify the effects of injecting RbCl solution on tree growth and to determine the uptake and distribution of Rb throughout the tree crown. Trees were randomly assigned a treatment, with three trees each for control, 8 g, and 16 g per tree of RbCl solution treatment groups. Methods of stem injection were the same as those described earlier for the sample trees in Amqui. Tree injections were carried out on 16 May 2014, to coincide with balsam fir bud burst in Fredericton. Branch samples were collected every week from 23 May to 18 July in the upper and lower crown of each tree. Branch samples were placed in brown paper bags labeled with tree number and date and transported back to the CFS lab for processing. The length of 25 current-year shoots from each branch was measured to the nearest 0.1 mm. These 25 shoots were placed in labeled bags and frozen at −20°C for subsequent Rb analysis.

Rubidium Analysis

The analysis to determine the concentrations of Rb in foliage, spruce budworm adults, and eggs was carried out by the Research and Productivity Council, Fredericton, NB, which is accredited by the Standards Council of Canada and is International Standards Organization (ISO) 9001:2008 certified.

Spruce Budworm Samples and Current-Year Shoots

From the laboratory diet experiment, a total of 18 spruce budworm adults (six from each treatment) and nine egg samples dissected from nine females (three from each treatment) were analyzed for Rb concentrations. From the field experiment in Amqui, 372 feral spruce budworm adults (91, 145, and 136 from control, 8 g, and 16 g per tree, respectively) that emerged from pupae collected from branch samples as well as 60 egg masses (20 randomly selected for each of the three treatments) were analyzed for Rb concentrations. In total, 468 terminal current-year shoots (156 for each treatment), collected weekly from the sample trees in the UNB woodlot, were analyzed for Rb concentrations.

Analysis of Samples for Rubidium

The methods outlined in this section apply to the foliage, spruce budworm adults, and egg samples. Foliage samples were dried in a low-temperature (35°C) oven for 3 d, with adult and egg samples being analyzed without removing any moisture. Sample weights ranged from 1.4 mg up to 460 mg.

Sample Digestion

Microwave-assisted digestion of tissue samples is generally described by Environmental Protection Agency (EPA) Method 3051A. Briefly, samples were prepared using microwave-assisted digestion in Fisher-brand trace metal grade nitric acid. Samples were accurately weighed into polytetrafluoroethylene microwave digestion pressure vessels. Nitric acid (7 ml) was added to each vessel, and the samples were heated in a Teflon-coated, electrically heated, graphite digestion block (110°C, ∼20 min). This predigestion step reduced the likelihood of excessive reaction and gas evolution during subsequent microwave digestion. The digestion vessels were sealed and placed into the carousel rack of the CEM Mars Xpress microwave digestion system (CEM Corporation, Matthews, NC). The microwave program includes a 5-min ramp to temperature (185°C). Temperature was held for 15 min, followed by a cool-down cycle. After cooling, the microwave vessels were opened, and the contents were quantitatively transferred into 50-ml flat-bottomed graduated polypropylene digestion tubes. The tubes were placed into the digestion block (110°C), and the solution volumes were reduced to ∼2 ml. After cooling, the solutions were diluted to volume (either 20 ml or 50 ml) and mixed thoroughly. Each sample preparation contained analytical reagent blanks, certified reference material, and analytical replicates (if sufficient material was available). The quality control samples were prepared and analyzed concurrently with the samples.

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Sample Analysis

EPA Method 200.8 was the general reference used for the inductively coupled plasma-mass spectrometry (ICP-MS) analysis of prepared solution samples. The solutions were diluted with 1% nitric acid (× 2 or × 5) before analysis for rubidium by ICP-MS. A Thermo Series II ICP-MS (ThermoFisher Scientific, Waltham, MA) instrument was used for all determinations. The dilution level chosen for each sample type was based upon sample weight availability, preparation volume, and the results of prescreening selected samples to determine approximate Rb concentrations. Some higher level samples required additional dilution to put the samples within the validated calibration range. Although rubidium has two major stable isotopes (^{85}Rb [72.2%] and ^{87}Rb [27.8%]), the ^{85}Rb was used for all analyses because of a potential isobaric interference from ^{87}Sr. An internal standard, ^{103}Rh, was used to moderate effects relating to sample transport and ionization due to varying amounts of dissolved solids in the analytical solutions. All solution standards were prepared by serial dilution of commercial 1,000 mg/liter stock solutions (SCP Science, Baie-d'Urfé, QC).

Quality control consisted of reagent blanks (RB), standard reference materials (SRM; NIST 1566b oyster tissue; NIST 1575 pine needles and NIST 2976 mussel tissue), and laboratory analytical duplicates. Samples were prepared and analyzed in four separate sets. Each microwave run included at least two RB and two SRM plus a maximum of 36 samples.

Statistical Analysis

Laboratory Experiments

The statistical analyses of data from all laboratory experiments are mentioned in the Results where appropriate.

Field Experiments

Differences in the Rb concentrations in the current-year foliage of trees injected with 8 and 16 g per tree and control trees, and between crown levels were tested using a repeated-measures analysis of variance (ANOVA) with crown level nested within tree (proc MIXED, SAS software, version 9.2). The analysis was performed on the mean Rb concentration per treatment per crown level per collection day. To determine the effects of different Rb concentrations on shoot growth, the mean growth of current-year shoots on all trees per treatment from the beginning to the end of the growing season were analyzed with one-way ANOVA. A GLM with treatment and sex as main factors was used to analyze differences in Rb concentrations in feral adults from the field. Differences in Rb concentrations in eggs collected from treatment and control trees were tested using one-way ANOVA.

All data from both laboratory and field experiments that were analyzed using either GLM or one-way ANOVA were first tested for normality using the Anderson–Darling test and, if necessary, subjected to either Box–Cox transformation or Johnson transformation. Post hoc multiple comparisons were conducted using Tukey's tests. With the exception of the repeated-measures ANOVA above, all statistical analyses were performed using Minitab16. A P value of 0.05 was used for all statistical analyses.

Results

Rubidium in Laboratory-Reared Spruce Budworm

Compared with the control diet, survival of L_2 during development to L_6, pupae, and adults was not affected by ingestion of the 1,000 µg/g RbCl diet (chi-square goodness-of-fit tests; all P values for each development period > 0.5), but was significantly reduced at each developmental period by ingestion of the 10,000 µg/g RbCl diet (chi-square goodness-of-fit tests; all P values < 0.001; Table 2). Treatments had a significant effect on the development times of both males and females from L_2 to L_6 (one-way ANOVA: $F_{5, 372} = 51.06$, $P < 0.0001$), from L_6 to pupae (one-way ANOVA: $F_{5, 372} = 100.62$, $P < 0.0001$), and from pupae to adults (one-way ANOVA: $F_{5, 349} = 15.74$, $P < 0.0001$). Within each developmental period, the developmental times of both males and females were significantly increased only by the 10,000 µg/g treatment (Tukey's test, Table 3). Although female pupae were significantly heavier than males regardless of treatment, only the weights of those males and females that ingested the 10,000 µg/g RbCl diet were significantly lower than those from the control and 1,000 µg/g RbCl diet treatments (one-way ANOVA: $F_{5, 372} = 217.27$, $P < 0.005$, followed by Tukey's test; Fig. 1). Sex ratios (% females) of pupae reared on both the 1,000 µg/g RbCl diet and the 10,000 µg/g RbCl diet were significantly more biased toward males compared with those reared on the control diet (chi-square goodness-of-fit tests; all P values < 0.05; Table 4). However, only the sex ratio of adults reared on the 10,000 µg/g RbCl diet became significantly more male biased compared with those reared on the control diet (chi-square goodness-of-fit tests; all P values < 0.05; Table 4). Mating success of females reared on the 10,000 µg/g RbCl diet was significantly reduced (chi-square goodness-of-fit tests; all P values < 0.05; Table 4) compared with the percentage of females that mated when reared on control diet.

Rb concentrations found in eggs and adult males and females differed significantly among treatments (one-way ANOVA; $F_{8, 81} = 753.44$, $P < 0.001$), with the highest concentrations found in eggs and adults reared on the 10,000 µg/g RbCl diet (Fig. 2). Only trace amounts of Rb were detected in male and female adults and eggs reared on the control diet. There were no differences in the Rb concentrations found in male and female adults and in eggs within each treatment (Tukey's test, Fig. 2).

Rubidium in Feral Spruce Budworm

Analysis of Rb concentrations in adults gathered from sample trees showed a significant effect of treatment (GLM; $F_{2, 310} = 390.71$, $P < 0.001$); Rb concentrations were significantly higher in adults from the 16 g per tree treatment than in adults from the 8 g per tree treatment, whereas Rb concentrations in adults from both treatments were significantly higher than in adults in the control (Tukey's test, Fig. 3). There was no difference in Rb concentrations

Table 2. Effects of rubidium (Rb) incorporated into artificial diet at concentrations of 1,000 and 10,000 µg/g RbCl on the percentage survival of spruce budworm from L_2 to L_6, to pupation, and to the adult stage

Development stage	Treatment	Survival %	n	χ^2
6th instar	Control	97.8	139	–
	1,000 µg/g	97.1	140	0.281
	10,000 µg/g	88.6	140	55.416*
Pupae	Control	95.7	139	–
	1,000 µg/g	95.7	140	0.0001
	10,000 µg/g	82.9	140	56.114*
Adult	Control	92.8	139	–
	1,000 µg/g	91.4	140	0.394
	10,000 µg/g	70.0	140	108.922*

*Significantly different from the control (all df = 1; $P < 0.05$).

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between males and females ($F_{1, 310} = 2.88$, $P = 0.09$), and there was no significant interaction between treatment and sex ($F_{2, 310} = 1.34$, $P = 0.264$).

There were significant differences in Rb concentrations in eggs collected from control and treatment trees (one-way ANOVA; $F_{2, 57} = 9.31$, $P = 0.001$). Rb concentrations in eggs from both the 16 g per tree treatment (8.90 [μg/g] ± 2.70; mean ± SE) and the 8 g per tree treatment (5.30 [μg/g] ± 1.35) were significantly higher than the control (1.28 [μg/g] ± 0.46), but there was no significant difference between the 8 and 16 g per tree treatments (Tukey's test).

There were no significant differences in pupal weight between spruce budworm that fed on treated trees and spruce budworm that fed on control trees (GLM; $F_{2, 310} = 1.75$, $P = 0.176$). However, there was a significant difference in pupal weight between males and females, with females weighing an average of 13 mg more than males ($F_{1, 310} = 71.15$, $P < 0.005$). The interaction between treatment and sex was not significant ($F_{2, 310} = 0.72$, $P = 0.488$). The size difference between male and female spruce budworm pupae is primarily due to sexual dimorphism (Blais 1952, Harvey 1957, Koller and Leonard 1981).

Based on the development of spruce budworm larvae in the sample taken on 24 June 2014 (the start of the pupal stage), there was a significant treatment effect on larval development (one-way ANOVA: $F_{2, 146} = 63.37$, $P = 0.004$). However, there were no significant differences between the development stage of larvae on the control trees (average instar of 5.5) and on the 8 g (average instar of 5.8) and 16 g (average instar of 5.4) per tree treatments, but a significant difference between the 8 and 16 g per tree treatments (Tukey's test).

Table 3. Effects of rubidium (Rb) incorporated into artificial diet at concentrations of 1,000 and 10,000 μg/g RbCl on the development time of spruce budworm from L_2 to L_6, from L_6 to pupation, and from pupation to adult stage

Sex	Treatment	Development time (d ± SE)		
		L_2 to L_6	L_6 to Pupae	Pupae to adults
Male	Control	10.20 ± 0.09a	5.18 ± 0.12a	7.40 ± 0.07ab
	1,000 μg/g	10.00 ± 0.00a	5.05 ± 0.09a	7.40 ± 0.06ab
	10,000 μg/g	10.99 ± 0.18b	6.79 ± 0.11b	7.91 ± 0.07c
Female	Control	10.16 ± 0.07a	7.02 ± 0.11bc	7.20 ± 0.05b
	1,000 μg/g	10.04 ± 0.04a	7.29 ± 0.13c	7.18 ± 0.08b
	10,000 μg/g	12.44 ± 0.26c	8.02 ± 0.16d	7.63 ± 0.09ac

Means within a column followed by different letters differ significantly (Tukey's tests; $P < 0.05$).

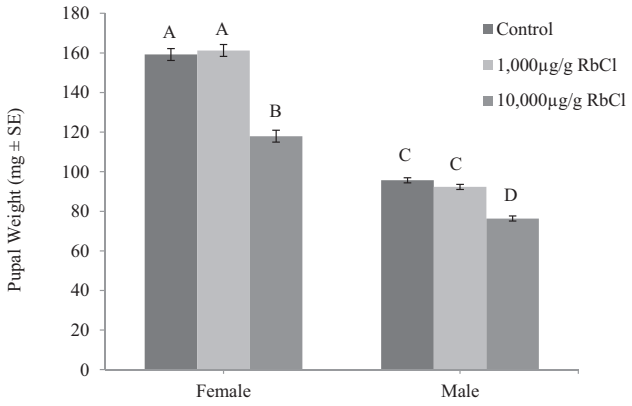

Fig. 1. Effects of rubidium (Rb) incorporated into artificial diet at concentrations of 1,000 and 10,000 μg/g RbCl on spruce budworm pupal weight (mean ± SE). Bars with the same letters are not significantly different ($P > 0.05$; Tukey's test).

Rubidium in Balsam Fir Trees

Background levels of Rb were present in the control trees at very low concentrations (Fig. 4). In the injected trees, the uptake of RbCl and translocation throughout the crown were relatively quick because Rb concentrations in the current-year foliage of injected trees were significantly higher than in the control trees only 1 wk after stem injection (ANOVA: $F_{2, 39} = 30.31$, $P < 0.005$; Fig. 4). Rb concentrations in the current-year foliage of injected trees continued to increase for about 3-wk postinjection, then declined slightly, but remained higher than the control trees over the 9-wk sampling period (Fig. 4).

Concentration levels of Rb in the trees were significantly affected by the injection of RbCl ($F_{2, 12} = 44.28$, $P < 0.001$), with both the 8 and the 16 g per tree treatments having significantly higher concentrations than the control trees (Tukey's test, $P < 0.05$; Fig. 4). Concentrations of Rb were similar in the lower and upper crowns ($F_{1, 12} = 0.30$, $P = 0.594$) for all treatments, thus, the interaction between treatment and crown level was not significant ($F_{2, 12} = 0.32$, $P = 0.735$).

At concentration levels of 8 and 16 g per tree, Rb did not affect the growth of current-year foliage; there were no significant differences between the length of current-year shoots on control trees and those on trees injected with RbCl (one-way ANOVA: $F_{2, 6} = 0.77$, $P = 0.504$).

Discussion

Rubidium in Laboratory Spruce Budworm

Our laboratory experiment showed that spruce budworm can be labeled with Rb when reared on artificial diet containing RbCl incorporated at concentrations of 1,000 and 10,000 μg/g. This is not

Table 4. Effects of rubidium (Rb) incorporated into artificial diet at concentrations of 1,000 and 10,000 μg/g RbCl on the sex ratio (% females) of spruce budworm pupae and adults and mating success (% mated) of female spruce budworm

Development stage	Treatment	Females (%)	*n*	χ^2	Mated (%)	*n*	χ^2
Pupa	Control	50.4	133	–	–	–	–
	1,000 μg/g	41.8	134	3.973*	–	–	–
	10,000 μg/g	38.7	111	6.038*	–	–	–
Adult	Control	51.1	129	–	85.7	56	–
	1,000 μg/g	43.8	128	2.843	80	50	1.326
	10,000 μg/g	40.8	98	4.229*	69.7	33	6.896*

*Significantly different from the control (all df = 1; $P < 0.05$).

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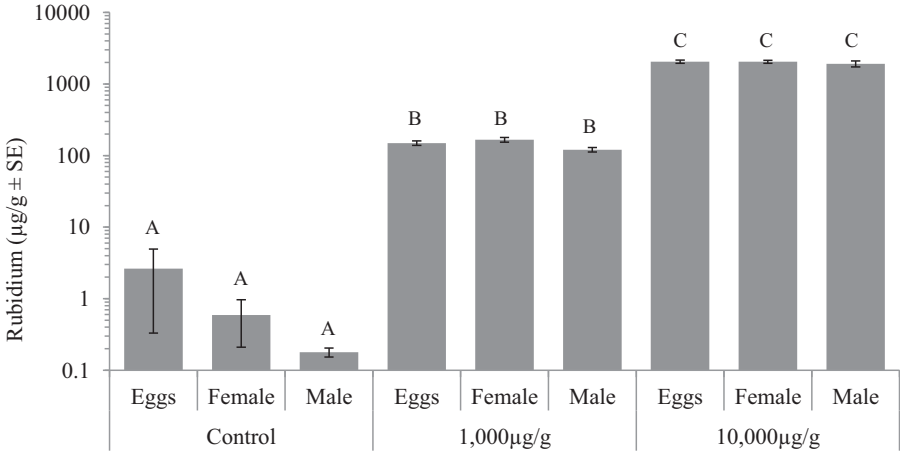

Fig. 2. Rubidium (Rb) concentrations (mean ± SE) found in male and female adults and eggs of spruce budworm reared on artificial diet (controls) and on artificial diets containing 1,000 and 10,000 µg/g RbCl. Bars with the same letter are not significantly different ($P > 0.05$; Tukey's test).

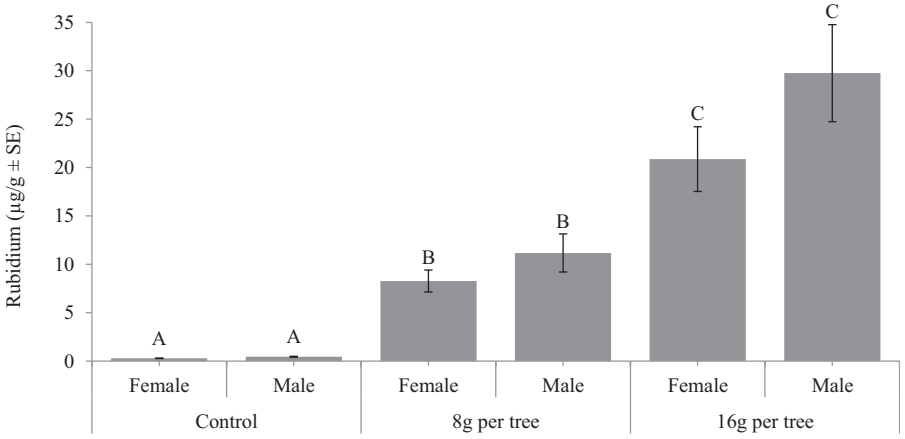

Fig. 3. Rubidium (Rb) concentrations (mean ± SE) found in male and female adults that fed as larvae on control trees and trees injected with 8 and 16 g per tree of RbCl solution. Bars with the same letter are not significantly different ($P > 0.05$; Tukey's test).

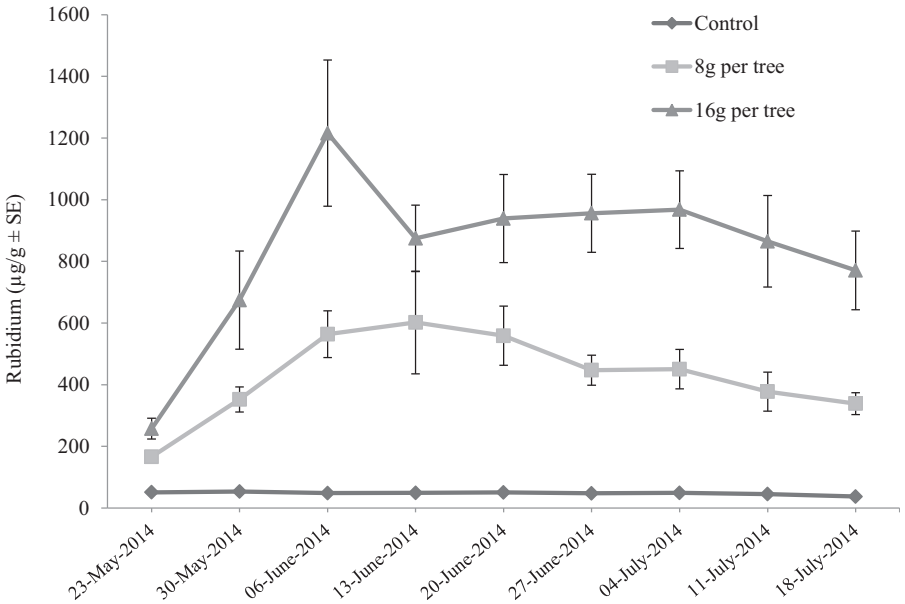

Fig. 4. Rubidium (Rb) concentrations (mean ± SE) found in the current-year foliage of balsam fir trees injected with 8 and 16 g per tree of RbCl solution on 16 May 2014 and in control trees in the UNB woodlot near Fredericton, NB.

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surprising, as various studies have shown that insects can be successfully labeled by rearing them on artificial diet containing various concentrations of RbCl (Jackson et al. 1988, Hopper and Woolson 1991, Qureshi et al. 2004a, Muratori et al. 2005, Ellis et al. 2012). Rb concentrations found in the adults and eggs were directly proportional to the dosage in the diet (Fig. 2), with specimens exposed to the 10,000 µg/g treatment possessing the highest concentrations. A directly proportional increase in Rb concentrations with dosage rate has also been found with the oriental fruit moth (*Grapholita molesta* Busck) (Ellis et al. 2012) and with the western tarnished plant bug (*Lygus hesperus* Knight) (Jackson et al. 1988).

We found that a low concentration of 1,000 µg/g RbCl incorporated into artificial diet had no significant effect on spruce budworm development or survival, whereas a high concentration of 10,000 µg/g RbCl resulted in significantly slower development time, lower survival rates, lower pupal weights in both males and females, male-biased sex ratio, and reduced mating success. Similar results have been observed in other studies. For example, Jackson et al. (1988) found no effects on the development of the western tarnished plant bug when reared on diet containing 100, 500, and 1,000 ppm of RbCl but observed significant negative effects at diet concentrations of 5,000 ppm. Qureshi et al. (2004a) found no significant effects on survival, pupal and adult weight, development time, and fecundity of the southwestern corn borer reared on diets containing RbCl and CsCl at 1,000 µg/g (1 g/liter). Similarly, Johnson and Reeves (1995) reported no effect on gypsy moth (*Lymantria dispar* L.) development or survival when reared on diet enriched with 3 g/liter of RbCl. We did not examine the effects of Rb on longevity and fecundity of the spruce budworm; however, Knight et al. (1989) found that tufted apple bud moth (*Platynota idaeusalis* Walker) reared on diet containing 3,000 µg/g showed no effects on fecundity or longevity, but at 6,000 µg/g, females died earlier and produced and deposited fewer eggs than those reared on control diet. The life span of oriental fruit moth adults was also shortened when reared on diet containing 6,000 ppm of RbCl (Ellis et al. 2012). Based on these results and our own study, it appears that low concentrations of RbCl incorporated into artificial diet can be used successfully to label the life stages of spruce budworm without affecting their life history.

Rubidium in Feral Spruce Budworm

Rb concentrations in the feral adults from control trees were very low, with an average of 0.4 µg/g to a maximum of 1.6 µg/g (Fig. 3). These background levels of Rb tend to exist everywhere in the environment (Berry and Smith 1969, Berry et al. 1972). Based on the standard set by Stimmann (1974), the adults from the 8 and 16 g per tree treatments were successfully labeled with Rb because concentration levels exceeded the mean (0.4 µg/g) of the control (male and female combined) plus three standard deviations (+0.78 µg/g). Furthermore, it is clear that adults that fed as larvae on trees injected with RbCl solution became labeled with Rb, as 100% of the feral adults that we analyzed had concentrations significantly higher than the control.

Eggs laid by feral female adults collected from labeled trees were also labeled with Rb. Interestingly, the ranges of Rb concentrations in eggs from both treatments and the control were large (range: 0.20–45.9 [µg/g], 0.30–20.7 [µg/g], and 0.20–9.20 [µg/g] for the 16 and 8 g per tree treatments and control, respectively). Also, the range of Rb concentrations in eggs collected from the control trees was greater than from females collected from the same control trees (0.20–9.20 [µg/g] vs. 0.10–0.70 [µg/g]). These field data suggest that there is lateral movement of moths among trees in the area, with moths from unlabeled trees laying eggs on treated trees and vice versa. Thus, moths do not restrict their egg laying to the trees on which they emerged.

There have been a number of studies in agricultural systems that have used RbCl and CsCl to label a variety of insect pests. For example, Qureshi et al. (2004b) successfully marked the southwestern corn borer by spraying the host plants with aqueous solutions of RbCl. The greenbug (*Schizaphis graminum* Rondani) was successfully labeled with Rb by spraying grain sorghum (*Sorghum bicolor* (L.) Moench) plants in the greenhouse with aqueous solutions of RbCl at concentrations of 2,500 and 10,000 ppm (Fernandes et al. 1997). Guillebeau et al. (1993) successfully labeled the green peach aphid (*Myzus persicae* Sulzar) with Rb for a period of 6 d following a spray application of RbCl solution on potted red pepper (*Capsicum annuum* Miller) plants at a rate of 10,000 ppm.

Compared with studies in agricultural systems, there are relatively few forest-based studies that have investigated the use of trace elements such as Rb and Cs to label defoliators such as spruce budworm. Kipp and Lonergan (1992) used a topical application of 0.41 M RbCl solution applied directly on male spruce budworm moths to successfully mark them in the field for at least 8 d with no adverse effects on mortality rates or on their ability to perceive female sex pheromones. McLean and Tuytel (1988) injected RbCl into the stems of Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) trees and found concentrations in the crown were potentially high enough to mark feeding western spruce budworm (*Choristoneura occidentalis* Freeman) based on lab experiments quantifying the percentage Rb uptake by larvae feeding on artificial diet (McLean and Laks 1985). Fleischer et al. (1989) injected RbCl into the stem of pin oak (*Quercus palustris* Muenchhausen) and found that a dosage of 200 g per tree was sufficient to label gypsy moth adults with Rb.

Our field experiments with feral spruce budworm populations showed that neither the 8 g nor the 16 g per tree treatment affected pupal weight and larval development times. There was a significant difference in larval development, but only between the two treatment blocks, with the development of larvae from the 16 g per tree block being slightly slower than that of larvae from the 8 g per tree block. However, neither treatment block was significantly different from the control. Minor microclimate differences between the blocks may have resulted in a slightly slower spruce budworm development rate in the 16 g per tree block because the tree canopy was slightly more dense than in the other two blocks—as a result, the tree crowns received less direct sunlight. As described earlier, the average instar difference between the two treatment blocks was only 0.4 of an instar (5.4 vs. 5.8), and neither treatment block was significantly different than the control block.

The mean Rb concentration averaged 125 µg/g for adults that were reared in the laboratory on 1,000 µg/g RbCl diet, with no effect on spruce budworm survival and development. This Rb concentration was approximately five times greater than that found in feral adults from the 16 g per tree treatment (25 µg/g) and 12 times higher than the 8 g per tree treatment (9 µg/g). Therefore, it is not unexpected that the comparably lower concentration levels injected into the trees would have no negative effects on spruce budworm survival and development in the field. Thus, we were able to successfully label spruce budworm adults in the field using low concentrations of RbCl without affecting their development rate and pupal weight.

Rubidium in Balsam Fir Trees

We found that RbCl was quickly absorbed and transported up the phloem of the balsam fir trees and remained at significantly higher

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levels in the current-year foliage of treated than control trees over the 9-wk sampling period (Fig. 4). McLean and Tuytel (1988) also reported that RbCl injected into the stem of Douglas fir trees was rapidly transported throughout the crown, with concentrations remaining at significantly high levels over a 6-wk sampling period. Fleischer et al. (1989) injected pin oak trees with 100, 200, and 500 g of RbCl and found that, after 4 d, the Rb concentrations significantly increased in the leaves to levels well above the control and remained high over a 23-d period. Fleischer et al. (1989) also found that the Rb concentrations in the leaves were directly proportional to the dose injected, which is similar to our findings of significantly higher concentrations in the trees injected with 16 g per tree compared with 8 g per tree (Fig. 4). We found no significant difference in Rb concentration throughout the crown of balsam fir. A similar result was reported for the distribution of RbCl throughout the crown of Douglas fir trees (McLean and Tuytel 1988). These results are not unexpected because Rb can be easily absorbed by plants as its chemical properties are very similar to K, and it can replace or partially replace K in living organisms (Stimmann et al. 1973).

Most studies that have investigated the use of trace elements to label insect populations have focused mainly on the effects on the insects and not the host plant. We found that injecting balsam fir trees with 8 and 16 g per tree of RbCl solution, concentrations well above the levels that naturally occur, did not have any negative effects on current-year shoot growth. Qureshi et al. (2004b) also reported similar results where spray applications of 100 and 1,000 ppm of RbCl did not have any negative effects on the growth of maize plants (*Zea mays* L.).

Overall, we successfully increased the concentration levels of Rb in the current-year foliage throughout the crown of balsam fir trees without causing any negative effects on tree growth. These concentration levels in the current-year foliage were sufficiently high to label feral-feeding spruce budworm adults and their eggs.

In conclusion, balsam fir trees injected with 8 and 16 g per tree of RbCl solution showed quick uptake and translocation throughout the current-year foliage in the crown. These concentrations had no negative effects on current-year shoot growth or on spruce budworm development. Adults that developed from larvae that fed on balsam fir trees injected with RbCl were clearly labeled because they had significantly higher concentrations than the background levels found in control trees. Rb concentrations in feral spruce budworm adults for both the 8 g (9 μg/g) and 16 g (25 μg/g) per tree treatments were at least five times lower than those in laboratory-reared adults on 1,000 μg/g RbCl diet (125 μg/g), which did not adversely affect the survival, development, pupal weight, sex ratio, or mating status of spruce budworm. Therefore, injecting balsam fir trees with concentration levels of 8 and 16 g per tree of RbCl is a viable technique for labeling feral spruce budworm adults and their eggs. Because moth migration can significantly influence posttreatment population assessment in early intervention treatment blocks, being able to label local spruce budworm may be very useful for distinguishing locals from immigrants to better evaluate the success of early intervention strategies such as pheromone mating disruption trials. Because the dosage of 8 g per tree was sufficient to clearly label spruce budworm adults and eggs, and because Rb is expensive, we recommend this dosage for use in large-scale research experiments.

Acknowledgments

We thank Joel Bates, Josh Sandwith, Rosanna Los, Melody McLean, Katie Burgess, Spencer Watts, Eric Garcelon, and Gaëtan LeClair for help with laboratory and field work. This research was funded by the Atlantic Canada Opportunities Agency (ACOA) project "Early Intervention Strategies to Suppress a Spruce Budworm Outbreak" and by Natural Resources Canada – Canadian Forest Service. All experiments reported here comply with the laws of Canada.

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