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Use of Rubidium to Label *Lysiphlebus testaceipes* (Hymenoptera: Braconidae), a Parasitoid of Greenbugs (Homoptera: Aphididae), for Dispersal Studies

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ABSTRACT A reliable method of labeling is needed to study dispersal of the braconid parasitoid, *Lysiphlebus testaceipes* (Cresson), an important biological control of greenbugs, *Schizaphis graminum* (Rondani), on grain sorghum, *Sorghum bicolor* (L.) Moench. The feasibility of using aqueous solutions of rubidium chloride (RbCl) applied as a foliar spray or soil drench to label greenbugs and *L. testaceipes* developing within greenbugs was studied. Laboratory and field studies were conducted to identify the minimal concentration of RbCl to assure labeling of greenbugs and wasps, persistence of Rb throughout the wasp's life span, mobility of Rb to unsprayed sorghum leaves, and feasibility of studying dispersal using a release-recapture technique with Rb-labeled wasps. Both greenbugs and wasps could be labeled using RbCl at concentrations of 2,500-10,000 ppm. Rubidium content in labeled wasps did not significantly vary during the first 7 d after emergence. Greenbugs feeding on unsprayed leaves were labeled up to 4 wk after leaves were sprayed. Rb-labeled wasps were found at the maximum trap distance from the release site (60 m) within 1 d after release.

KEY WORDS *Lysiphlebus testaceipes*, greenbugs, parasitoids, dispersal, rubidium chloride, grain sorghum

DESPITE THE IMPORTANCE of parasitoids for biological control of pests, studies of their dispersal are rare. Release-recapture techniques often have been used for insect dispersal studies (Southwood 1978). According to Akey (1991), markers should be easy to apply, require minimal manipulation, be easily recognized, persist with certainty, and be without deleterious biological effects to the recipient.

The use of trace elements such as rubidium (Rb), cesium (Cs), and strontium (Sr) has been one of the best approaches to label insects (Hopper 1991, Stimmann 1991). Elevated levels of trace elements allows discrimination of wild and labeled individuals. The concentration of a trace element has been increased in insects by incorporating it into artificial diets or by spraying their host plants. Rb was first proposed as an internal physiological marker by Berry et al. (1972). These authors emphasized that Rb is an excellent marker because it is rare in nature and does not pose an environmental hazard (nonradioactive properties). This element also is easily absorbed by plants, which makes its use simple. Rb and other trace elements have been used to label herbivores, predators, and parasitoids (Frazer and Raworth 1974, Fleischer et al. 1986, Jackson and Debolt 1990, Jackson 1991). To label parasitoids, transferral of Rb from lower trophic levels is usually required because few parasitoids can be reared on artificial diets (Jackson 1991). Rb is eliminated from labeled insects by excretion, oviposition, and mating (Van Steenwyk 1991). The rate of excretion depends upon the insect's feeding habits and the stage in which Rb was acquired.

In addition to benefits such as ease of application under field conditions (Graham et al. 1978) and detection by techniques such as atomic absorption spectrophotometry and atomic emission spectrophotometry (Hopper 1991), Rb does not adversely affect the biology and behavior of insects under moderate concentrations (Stimmann et al. 1973, Graham and Wolfenbarger 1977). Larvae of pink bollworm, *Pectinophora gossypiella* (Saunders), fed on RbCl-supplemented artificial diet were not affected by concentrations of ≤85,500 ppm, beyond which high larval mortality was noticed (Van Steenwyk et al. 1978).

Several sorghum, *Sorghum bicolor* (L.) Moench, insects—*Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J. E. Smith), *Epitrix* spp., *Diabrotica* spp., Cicadellidae, Aphididae, Coccinelidae, Chrysopidae, and Syrphidae representing both herbivores and predators—have been labeled by spraying Rb on plants (Graham et al. 1978). Insects collected

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from treated plants showed higher amounts of Rb 28 d after plants were sprayed. There is no reference in that study regarding effects on parasitoids.

Dispersal studies can be conducted by using labeled insects which are released, recaptured, and identified. According to Hayes (1991), traps distributed along concentric circles around the release site and frequent sampling are the best approach for dispersal studies of insects when dispersal behavior is not well known. Limited previous research indicated that *L. testaceipes* did not disperse more than a few meters from the release site (Starks et al. 1973).

Labeling of *Lysiphlebus testaceipes* (Cresson), an important parasitoid of greenbugs, *Schizaphis graminum* (Rondani), in Nebraska, is essential if its dispersal is to be studied. Thus, the ability to apply and detect Rb accurately in parasitoids must be evaluated and analyzed on an individual insect basis to allow separation of labeled and unlabeled parasitoids. The objectives of this study were as follows: (1) to determine if Rb could be detected in both greenbugs, and its parasitoid, *L. testaceipes*, after application to plants; (2) to determine the mobility of Rb in sorghum plants; and (3) to test the feasibility of using a release–recapture method to measure dispersal of Rb-labeled *L. testaceipes* under field conditions.

Materials and Methods

Rb Detection in Greenbugs and Parasitoids. Seeds of a biotype-E greenbug susceptible sorghum hybrid (Funk 522 DR) were sown in 200-ml plastic pots filled with a mixture of soil, sand, and vermiculite (≈10:2.5:1). After emergence, plants were thinned to 1 per pot. These plants were maintained in a greenhouse for 4 wk, after which they were transferred to the laboratory (room temperature). Plants were watered once a week.

The sorghum seedlings (4 wk old) were treated with solutions of RbCl—either 0; 500; 1,000; 2,500; 5,000; or 10,000 ppm of RbCl sprayed on plants or 10,000 ppm of RbCl applied as a soil drench. The experimental design was completely randomized with 5 replications (plants). The spray solutions were prepared with distilled water, RbCl (Sigma, St. Louis, MO), and 0.5 ml of a wetting agent (Activator 90; Loveland Industries, Loveland, CO). These solutions were applied to plants using a 1-liter garden sprayer. The treatment with 10,000-ppm soil drench was prepared as was the spray solutions but without the wetting agent. The control treatment was prepared with all ingredients except RbCl. Plants were sprayed thoroughly to runoff and allowed to dry for 24 h. About 20 ml of solution per plant was used for both treatment methods.

Thirty parasitoids (sex ratio 1:1) were placed in a cage with ≈100 laboratory-reared biotype E greenbugs for 24 h. Plants were then infested with 5 greenbugs that had been exposed to parasitoids for 24 h. Aphids were transferred to plants using a camel's-hair brush. Plants were caged and maintained at room temperature. After ≈5 d, parasitized aphids became mummies. Each mummy was carefully removed from the plants and isolated in gelatine capsules (Eli Lilly, Indianapolis, IN) to allow individual parasitoids to emerge. Emergence occurred after 3–4 d. Adults were immediately transferred to a freezer (−10°C) for a few minutes and then transferred to clean microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA). These tubes were kept in the freezer at −10°C for later analysis of Rb content. Some unparasitized greenbugs were also kept in the freezer for analysis.

Rubidium Analysis. Insects were individually placed in 5-ml acid-washed Teflon bombs. To each bomb, 0.5 ml of trace element grade hydrochloric acid and 0.5 ml of trace element grade nitric acid was added. Bombs were sealed and heated at 80°C for 24 h. Bombs were cooled, and the digested sample solutions were transferred to acid-washed 25-ml volumetric flasks. Indium was added to the sample solutions as an internal standard and the flasks were brought up to volume with ultra-pure (18 M ohm/cm) water. This resulted in an insect solution concentration of 1–4 ppm insect (depending on insect weight) with a 50-ppb indium internal standard spike. Because individual insects had too little mass for accurate dry-weight measurement, insect weight was estimated by weighing a large number of insects and calculating an average dry weight for aphids (0.13 mg) and wasps (0.026 mg). Rubidium-standard solutions of 1, 10, and 100 ppb were prepared using trace element grade rubidium chloride and ultra-pure water. Rubidium content of each solution was analyzed using ICP Spectroscopy (VG Plasmaquad ICP-MS, Danvers, MA). Five scans of each standard solution and 3 scans of each sample solution were done using an aliquot of ≈1 ml per scan. Rubidium content of each sample was calculated by averaging the results of the 3 sample scans. Rubidium content of each insect was calculated from the insect solution concentration.

Insects were considered labeled if they exceeded at least 3 standard deviations from the mean background level of insects feeding on untreated plants (Stimmann 1974). Dose–response curves for both greenbugs and parasitoids were described by polynomial regression analysis (SAS Institute 1990).

Rb Detection Throughout Parasitoid's Life Span. Sorghum seedlings and parasitized greenbugs were obtained as described above, but parasitoids had been reared in greenbugs on field-caged sorghum for a few generations before use in this study. A solution of 5,000 ppm RbCl was prepared and sprayed on all plants. Plants were covered with plastic tube cages (Morgan et al. 1980) in the laboratory and held at room temperature. Plants were infested with 5 unparasitized greenbugs per plant 24 h after spraying. Approximately 1 wk after greenbug infestation, newly emerged parasitoids were placed in the cages. A randomized complete block design with 4 replications was used. Blocks con-

sisted of different parasitoid introduction dates because of irregular parasitoid emergence. Only 1 parasitoid was kept per cage in 3 replications. A 4th block was conducted with parasitoid pairs (1 male and 1 female) rather than with individuals in the cage. Parasitoids were removed from cages and frozen at −10°C on 1, 2, 3, 4, 5, 6, and 7 d after emergence. Sample preparation and Rb analysis were performed similarly to the previous experiment. Analysis of variance (ANOVA) for a randomized complete block design was conducted (SAS Institute 1990) to test whether rubidium content varied significantly with time after wasp emergence.

Rb Mobility in Sorghum Plants. Sorghum plants were maintained similarly to the 1st experiment, but 700-ml clay pots were used instead of plastic pots. Seedlings were thinned to 1 plant per pot. Pots were transferred to the laboratory when plants had 3 fully developed leaves. Pots were watered twice a week. Half of the plants were randomly selected and sprayed with 5,000 ppm RbCl solution to runoff as described previously. The check consisted of plants sprayed with the same solution without RbCl. Seven replications were used. Plants were infested with greenbugs 25 d after treatments, after new unsprayed leaves had emerged. Each plant was infested with 2 adult greenbugs, 1 on the 3rd leaf (previously treated) and the other on the 6th leaf (emerged after treatment). Aphids were caged on these leaves using a small 1-sided leaf cage made from adhesive foam mounting (Lundell Manufacturing, Minneapolis, MN) with an opening (5 mm diameter) covered with muslin. Aphids were removed 1 wk later and transferred to clean plastic microcentrifuge tubes and held at −10°C. The leaves on which the aphids were caged were removed, dried for 30 d at room temperature, weighed, and transferred to clean plastic microcentrifuge tubes. Greenbug and leaf Rb content were analyzed as described above, but they were digested in a solution of 1.0 ml trace element grade hydrochloric acid and 1.0 ml trace element grade nitric acid for 24 h. ANOVA was conducted with leaves as subplots and Rb treatments as main plots in a split plot design (SAS Institute 1990).

Release–Recapture Study. A 2-ha sorghum field located near Mead (Saunders County), NE, 48 km N of Lincoln, NE, was used. Sorghum (Dekalb 42Y) plants were in stage 5 (boot stage) (Vanderlip 1972). One 8-m^3 saran fabric (32 by 32 mesh) cage (Bioquip Products, Gardena, CA) was placed in the center of the study area. Sorghum plants in the cage were artificially infested with large numbers of greenbugs. Two weeks later, plants were sprayed with a 5,000-ppm RbCl solution prepared in the same way as the laboratory experiments. Plants were throughly sprayed to runoff using a hand sprayer. Parasitoids (≈150 adults) were released in the cage immediately after spraying. Eleven days after parasitoid release, 350 aphid mummies were observed. Before removal of the cage, 81 white sticky Pherocon 1C trap liners (Trécé, Salinas, CA) were folded and placed on stakes (0.5 m tall) at 10-m intervals along concentric circles around the cage located at 5, 10, 20, 40, and 60 m from the cage (Fig. 1). A caged plastic pot containing greenbug-infested sorghum seedlings was kept beside each trap to assure even infestation of the field and, therefore, attractiveness (Grasswitz and Paine 1992) because of very low levels of greenbugs or mummies in the field at the time of wasp release. Another sticky trap was placed in the center of the area covered by the field cage for 2 d.

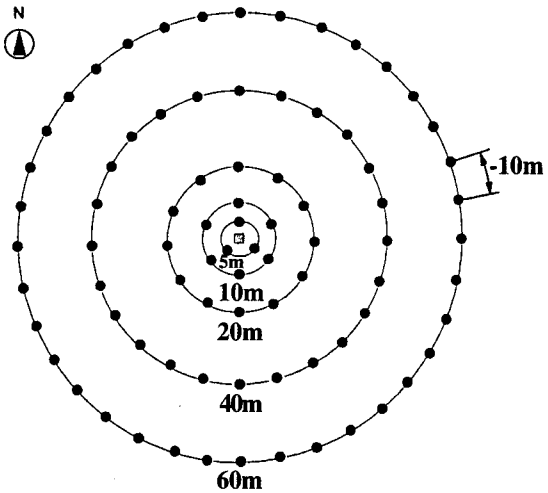

Fig. 1. Distribution of sentinel traps (sticky traps adjacent to caged greenbug-infested, potted sorghum plants) used to monitor *L. testaceipes* dispersal in a commercial grain sorghum field.

Sticky traps were replaced daily over a 5-d period and held at 5°C. Captured parasitoids were removed from traps, identified as *Lysiphlebus* spp. (Johnson et al. 1979), and transferred directly to clean 25-ml bombs for Rb content analysis. The analytical procedures were as described above.

Results

Rb Detection in Greenbugs and Parasitoids. The relationship between the concentration of RbCl applied on plants and Rb concentration detected in both greenbugs and parasitoids indicated a significant quadratic trend (Fig. 2). It also is noticeable that the concentration of Rb in parasitoids was slightly greater than in greenbugs at similar concentrations of applied Rb.

Greenbugs and *L. testaceipes* reared on sorghum plants not treated with RbCl had Rb contents (ng/mg of dry body weight) of 27.56 ± 5.87 (mean ± SD), and 73.08 ± 32.32, respectively.

According to Stimmann (1974), an insect is considered to be labeled if trace element concentrations are >3 SDs above the mean background level. Thus, in this study, greenbugs and *L. testaceipes* were considered labeled if they exceeded 45.17 and 170.04 ng of Rb/mg of dry body weight, respec-

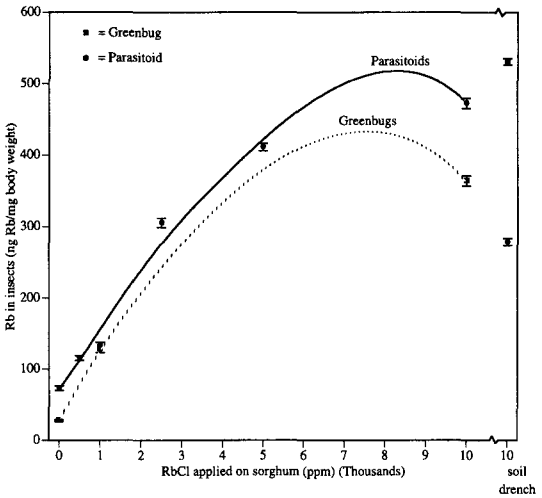

Fig. 2. Relationship between concentration of Rb (y) (ng Rb/mg body weight [mean ± SE]) detected in greenbugs and parasitoids and concentration of RbCl (ppm) sprayed on sorghum plants (x). Regression equations are as follows: greenbugs; $y = 27.56\ (\pm\ 73.9) + 0.11\ (\pm\ 0.12)\ x - 7.66 \times 10^{-6}\ (\pm 1.1 \times 10^{-6})\ x^2$ ($R^2 = 0.64$; F = 5.445; df = 2, 6; $P = 0.045$); parasitoid; $y = 62.03\ (\pm 30.86) + 0.10\ (\pm 0.02)\ x - 6.05 \times 10^{-6}\ (\pm\ 2.01 \times 10^{-6})\ x^2$ ($R^2 = 0.75$; F = 35.73; df = 2, 24; $P < 0.001$). The soil drench data points were not used in developing the regression equations.

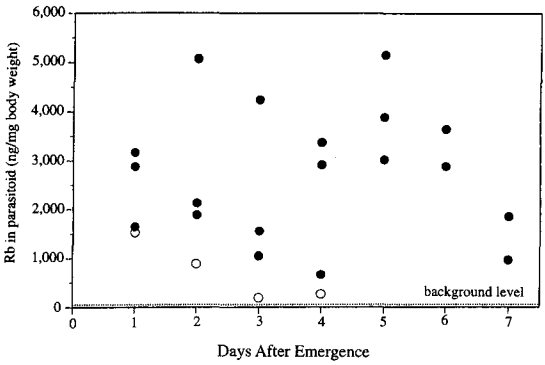

Fig. 3. Amount of rubidium (ng Rb/mg body weight) in individual parasitoids throughout their life span compared with background level (52.60 ng Rb/mg body weight). Regression of rubidium content of parasitoids over time was not significant for either linear or quadratic components ($P > 0.1$). ○, individuals held as pairs (1 male and 1 female); ●, wasps maintained individually.

tively. According to these criteria, foliar sprays of 2,500 ppm of RbCl should be the minimal level for labeling *L. testaceipes*, and greenbugs should be labeled using foliar sprays of at least 1,000 ppm of RbCl. Greenbugs and wasps caged on plants treated by a soil drench of 10,000 ppm RbCl also were labeled; they contained 530.77 ± 60.60 ng of Rb/mg of dry body weight and 278.85 ± 76.02, respectively.

Rb Detection Throughout Parasitoid's Life Span. Analysis of variance indicated no significant variation ($F = 0.02$; df = 1, 20; $P = 0.895$) in the amount of Rb during the parasitoid's life span (Fig. 3). However, there was a significant block effect ($F = 7.97$; df = 1, 20; $P = 0.011$). The block composed of pairs of parasitoids (1 male and 1 female) had declining Rb content with time. Also, although the insects used in this experiment belonged to the same laboratory colony used in the previous experiments, the amount of Rb in the parasitoids was much higher than detected during the 1st experiment (Fig. 2).

Rb Mobility in Sorghum Plants. Analysis of variance of the mean amount of Rb detected from both leaves shows that the treatment did not significantly increase ($F = 5.73$; df = 1, 2; $P = 0.139$) the Rb content in leaves. However, there was a significant interaction effect (rubidium × leaf) ($F = 60.96$; df = 1, 20; $P < 0.001$); the amount of Rb in 3rd and 6th leaves were not significantly different ($F = 0.00$; df = 1, 20; $P = 0.999$) in the check but they were significantly different ($F = 121.93$; df = 1, 20; $P < 0.001$) when Rb was sprayed. The younger leaf (leaf 6) contained less Rb compared with the older one (leaf 3) (Table 1). However, despite this, greenbug rubidium content was significantly influenced only by the rubidium treatment ($F = 6.28$; df = 1, 17; $P = 0.023$), and greenbugs were labeled regardless of whether they fed on leaf 3 (previously sprayed with rubidium) or leaf 6 (emerged after rubididum treatment) (Table 1).

Release–Recapture Study. In total, 19 parasitoids were caught by the sticky traps during the study period. Five parasitoids were caught at the 0-m location (cage area) and 14 were caught by the sticky traps placed around the release site. However, 1 parasitoid could not be retrieved from the sticky trap and only 18 insects were analyzed for Rb content. All trapped parasitoids contained high amounts of Rb (980.76–1519.23 ng Rb/mg of body weight) when compared with the background level detected in nonlabeled insects. Therefore, we assume that all 18 parasitoids trapped and analyzed were labeled by RbCl sprayed on plants at the release site. They were able to spread several meters on the first 2 d after release (Fig. 4), and even the most distant traps caught several individuals. The wind direction and speed might have influenced the dispersal of the parasitoid because, overall, there was a relationship between wind direction and parasitoid flight directions; most movement appeared to be downwind (Fernandes 1995). Natural infesta-

Table 1. Mean (±SE) amount (ng/mg of dry matter) of Rb detected in greenbugs and seedling sorghum leaves

Sorghum leaf	Amt of Rb in leaf		Amt of Rb in greenbug	
	Untreated	RbCl-treated	Untreated	RbCl-treated
3rd	74.31 (±18.73)	7,850.97 (±796.16)	6.09 (±0.77)	80.38 (±36.27)
6th	74.22 (±22.27)	1,576.00 (±417.27)	11.86 (±3.89)	25.96 (±4.34)

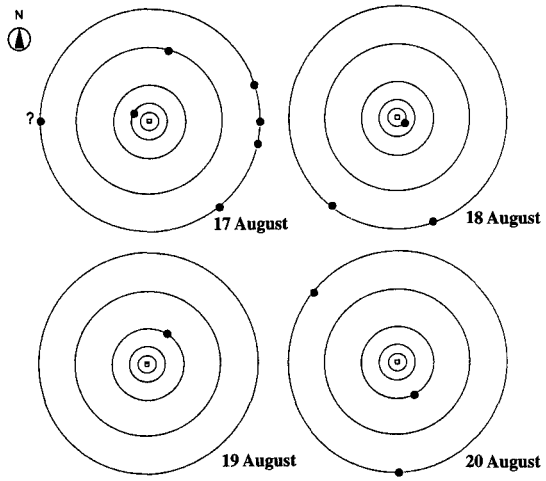

Fig. 4. Location of parasitoids captured by sticky traps in release–recapture study using Rb-labeled wasps. Circles represent 5, 10, 20, 40, and 60 m from central release site (see Fig. 1). Each solid circle represents 1 labeled parasitoid caught, based on later analysis of Rb content. ?, location of a trapped parasitoid whose Rb content was not analyzed.

tions of aphids and parasitoids were scarce in the field during the study.

Discussion

This study demonstrates that both *L. testaceipes* and greenbugs can be labeled with Rb when sorghum plants were sprayed to runoff with concentrations of RbCl >2,500 ppm. Parasitoid labeling with Rb was also accomplished by Corbett et al (1996), Jackson et al. (1988), Jackson and Debolt (1990), and Hopper (1991) with other hymenopteran species, whereas Guillebeau et al. (1993) labeled another aphid species.

Rubidium content in new, unsprayed leaves of plants sprayed 32 d earlier was higher than in leaves from untreated plants. This shows that Rb is mobile within the plant. Greenbugs feeding on the new, unsprayed leaves obtained sufficient Rb to become labeled (Table 1). Therefore, greenbugs could be labeled up to 4 wk after spraying the plants. Although, parasitized greenbugs were not tested during the Rb mobility assay, the 1st experiment showed that the Rb content of both greenbugs and parasitoids were very similar (Fig. 3). Therefore, we speculate that parasitoids would be labeled if they also developed within this period. As Levi (1968, 1970) has shown, Rb can replace K and is also highly mobile in plants. Levi studied the mobility of Rb and observed a rapid translocation to new leaves within a few hours following treatment. Therefore, this should result in labeling, even though insects may appear on a plant several days after treatment.

This is the 1st report of relatively long-distance dispersal by *L. testaceipes*. This parasitoid moved at least 60 m in 1 d, which is much farther than previously reported (Starks et al. 1973) These results indicate that Rb can be used as a physiological marker in release–recapture studies of *L. testaceipes* under field conditions. Additional studies of *L. testaceipes* dispersal are needed to define its dispersal ability better. This information would be useful in developing strategies for augmentative releases of *L. testaceipes* as a biological control of greenbugs.

The use of sentinel traps (association of greenbug-infested plants and sticky traps) is feasible for *L. testaceipes* and probably other parasitoid dispersal studies. However, they may not be needed if studies are done in a field with abundant hosts. *Lysiphlebus testaceipes* remained labeled for 7 d after emergence, although the variability of the data was very high (Fig. 3). The amount of Rb observed in these insects was ≈10 times that in the previous experiment (Fig. 2). It is possible that the insects brought to the laboratory may exhibit different uptake and excretion rates. It also is possible that pairs of parasitoids or even groups may have trace element levels decreased at a much higher rate because of greater possibility of mating and oviposition. Jackson et al. (1988) used Rb to label *Anaphes ovijentatus* (Crosby & Leonard), an egg parasitoid of *Lygus* spp. The authors reported that the parasitoids fed on diets containing 500 or 1,000 ppm of RbCl were labeled up to 4 d after emergence. Corbett et al. (1996) reported minimal decay of Rb levels in labeled *Anagrus epos* Girault adults, but this mymarid did not live >72 h after adult emergence.

Although Akey and Burns (1991) did not report ICP spectroscopy to be among the best analytical procedures, in our study it was able to detect Rb with sufficient sensitivity to distinguish between natural and artificial levels of Rb in individual greenbugs and wasps. However, this technique is expensive. Each run (1 insect) cost approximately US$15. For larger numbers of insects, as would be needed for dispersal studies, atomic absorption spectrophotometry may be preferred because of cost considerations.

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