Labeling an Egg Parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae), with Rubidium within an Overwintering Refuge

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ABSTRACT French prune trees provide overwintering habitat for the egg parasitoid *Anagrus epos* Girault (Hymenoptera: Mymaridae), an important natural enemy of the the grape leafhopper, *Erythroneura elegantula* Osborn (Homoptera: Cicadellidae). French prune trees were treated with rubidium during the fall of 1991–1993 to assess the potential for obtaining an elemental label in overwintering *A. epos*. Multiple applications of 5,000-ppm solutions of RbCl to French prune foliage from late August to early October resulted in increases in foliar rubidium content of up to 200 times the naturally occuring concentration. Rubidium content of adult prune leafhoppers, *Edwardsiana prunicola* Edwards (Homoptera: Cicadellidae), collected from treated trees was up to 130 times the naturally occurring level. Naturally occuring rubidium content of *A. epos* was 0.052 ng Rb per individual based on a sample of 498 wasps collected from 3 separate, untreated grape vineyards. Mean rubidium content was 0.205 ng Rb in *A. epos* collected in the spring directly from French prune trees that had been treated in the fall with RbCl. This is 3.9 times the background level and indicates successful labeling of overwintering *A. epos*. However, there was substantial overlap in rubidium content between *A. epos* from treated trees and those from untreated sites. *A. epos* reared from rubidium-enriched host eggs and maintained in the lab on a mixture of honey and water exhibited minimal decay of the elemental label during the adult lifetime. Success of this labeling approach has made possible the direct study of the dispersal of *A. epos* from treated French prune tree refuges into adjacent grape vineyards.

KEY WORDS *Anagrus epos*, western grape leafhopper, grapes, elemental labeling, refugia, agricultural landscapes

INSECTS OFTEN COLONIZE crops from external habitats during the early period of crop growth. This colonization occurs either from established populations on annual crops that are in the middle of their growing season (Stern 1969, Brandenburg and Kennedy 1982, Fleischer et al. 1988, Corbett et al. 1991), or from native vegetation or perennial crops in which the insect overwinters (Doutt and Nakata 1973, Thomas et al. 1991). The amount of colonization experienced by an agricultural field depends in complicated ways on the nature of the surrounding agricultural landscape (for example, the proximity and size of potential sources of colonizers), on the dispersal behavior of the insect, as well as on climatological and physical aspects of the environment (for example, Lewis and Dibley 1970). In most cases, the source of colonizers of a particular crop is not easily determined, and without detailed study it is not necessarily clear what the contribution is by various surrounding landscape components (Brandenburg and Kennedy 1982, Bishop and Riechert 1990, Wratten and Thomas 1990). Elucidating the dynamics of dispersal and colonization, particularly from overwintering habitats, is critical to understanding regional population dynamics of both herbivores and natural enemies. It is especially important in the successful integration of overwintering refuges within annual cropping systems for the enhancement of natural enemies (Pickett et al. 1990, Landis and Haas 1992, Corbett in press). We present in this article a method for the direct study of early-season dispersal of a parasitic wasp from an overwintering refuge. This method involves the chemical labeling of overwintering wasps with the trace element rubidium, with the goal of their subsequent recapture in an adjacent crop following spring emergence.

Rubidium has been used successfully to label both herbivorous and entomophagous insects in the field (Payne and Wood 1984, Fleischer et al. 1988, Theony et al. 1992; see Akey et al. 1991 for a thorough review). Because of its location between potassium and cesium in the periodic table, rubidium behaves as a chemical analog to potassium; therefore, it readily replaces potassium in biological tissues (Berry et al. 1972). At low concen-

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trations, rubidium appears to have no detectable effects on survivorship or behavior (VanSteenwyk 1991); therefore, it can be used without disrupting the natural dispersal behavior of an insect. The amount of rubidium in biological samples can be determined relatively easily through the method of atomic emission spectrophotometry (that is, measuring the emission of element-specific wavelengths by excited atoms) (Akey and Burns 1991). Because rubidium occurs naturally in plant and insect tissues at fairly low and uniform levels (across geographic areas), relatively small increases in rubidium result in a reliable elemental label that is detectable above the natural background concentration.

Rubidium labeling has been achieved either through direct sprays of RbCl solution onto foliage of herbaceous plants (Graham et al. 1978, Wolfenbarger et al. 1982, Fleischer et al. 1988) or through injection of RbCl solutions into the xylem of woody plants (Fleischer et al. 1991, Theony et al. 1992). Herbivorous insects obtain a label through feeding on rubidium-enriched plant tissues. Predatory insects (for example, *Geocoris* spp. [Jackson 1991], *Orius* spp., and miscellaneous spiders [Graham et al. 1978]) obtain detectable labels through feeding on labeled herbivorous insects. Parasitic Hymenoptera also have been shown to obtain a rubidium label through development within a labeled host (Payne and Wood 1984, Jackson et al. 1988, Hopper and Woolson 1991).

Obtaining a detectable rubidium label in an insect emerging in spring requires the retention of the label through an overwintering stage, typically lasting many months. The longevity of a rubidium label (the time following removal from the Rb source during which a label is detectable above the background level) is largely dependent on the amount of time spent feeding on the source and the amount of feeding done after removal from (or dispersal from) the source (VanSteenwyk 1991). In principle, an insect in a quiescent, overwintering phase should retain the rubidium label because there will be no feeding, and loss of the Rb through some other route is unlikely. Results by Wolfenbarger et al. (1982) with the boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), support this proposal. They found detectable levels of rubidium in boll weevils in March, 6 mo following application of RbCl solution to cotton fields. They hypothesized that these individuals may have overwintered near the study field. Such long-term labeling should also be possible for a developing parasitic wasp using an overwintering herbivorous host. Evaluation of rubidium labeling of parasitic wasps has so far been done either in the laboratory or in the field within the same growing season as treatment with RbCl. To date there are no published accounts of attempts to label a parasitoid of an overwintering herbivorous host in the field.

Our research focuses on *Anagrus epos* Girault, an egg parasitoid of the western grape leafhopper, *Erythroneura elegantula* Osborn (Homoptera: Cicadellidae), an important pest of grapes in California. *A. epos* is a native species that attacks numerous leafhopper species; parasitism of grape leafhopper regularly approaches levels of ≥90%. *A. epos* overwinters within the egg stage of its host. Because grape leafhopper overwinters as an adult, *A. epos* cannot overwinter within vineyards but must instead colonize from external overwintering sites, particularly the overwintering eggs of leafhoppers using *Rubus* spp. within riparian habitats (Doutt and Nakata 1973). Kido et al. (1984) demonstrated that *A. epos* also overwinters in eggs of the prune leafhopper, *Edwardsiana prunicola* (Edwards) (Homoptera: Cicadellidae), on French prune trees. As a result, research has proceeded on the potential for use of French prune trees as overwintering refuges for *A. epos* within vineyard agroecosystems (Pickett et al. 1990, Murphy et al. in press). The research presented in this article was undertaken with the goal of using rubidium labeling to quantify the contribution of French prune tree refuges to early-season *A. epos* populations in adjacent vineyards and to study *A. epos* dispersal behavior following emergence. In this article, we focus on an evaluation of the feasibility of labeling *A. epos* overwintering in French prune trees. An analysis of recaptures of labeled individuals will be presented in subsequent articles.

Successful labeling of *A. epos* overwintering in French prune tree refuges for the study of dispersal and colonization requires the successful completion of a series of steps. Specifically, the following 4 questions need to be addressed: (1) Is it possible to enrich the rubidium content of French prune foliage late in the season, during the period when prune leafhopper females that will lay overwintering eggs are developing? (2) Will feeding by immature prune leafhoppers on rubidium-enriched foliage result in labeling of adults and their eggs? (3) Do *A. epos* that have overwintered in prune leafhoppers eggs in treated French prune trees contain levels of rubidium that are measurably greater than untreated *A. epos*? (4) Will *A. epos* with increased rubidium content retain the label over a significant portion of their life? In this article we present the results of an evaluation of these steps.

Materials and Methods

Study Sites. We applied rubidium to French prune trees during the fall of 1991, 1992, and 1993 to obtain labeled *A. epos* emerging the subsequent springs (1992, 1993, and 1994, respectively). In 1991, rubidium was applied at 2 sites at E. & J. Gallo Ranch near Livingston, CA (Merced County). The Hayes' site consisted of sixty, 2-yr-old French prune trees ≈5 m in height in 2 staggered rows. The D20 site consisted of fifty 2-yr-old trees

in a single row that were 4–6 m in height. At both sites the overwintering refuges were immediately adjacent to vineyard blocks (separated by no more than 3 m). Rubidium was applied at 2 sites again in 1992. The D20 site at E. & J. Gallo Ranch was used a 2nd time. The 2nd site was at Chalone Vineyards, ≈20 km east of Soledad, CA (Monterey County). This site consisted of forty-eight 3-yr-old trees in a 3 × 16 rectangular block that were ≈5 m in height. This refuge was separated from the adjacent vineyard block by 10 m. Rubidium was applied in the fall of 1993 at a single site ≈5 km south of Clements, CA (San Joaquin County). This site consisted of a large, well-established French prune orchard (≈900 trees) bordered on the north by a grape vineyard.

Application of Rubidium to French Prune Trees. We applied RbCl to French prune trees through direct sprays to foliage from late August to early October. Although trunk injection of a RbCl solution has become the common method for enriching rubidium concentration in trees because of its simplicity and effectiveness (Payne and Wood 1984, Fleischer et al. 1991, Theony et al. 1992), we opted to use foliar sprays because of the following factors: concern over the potential of injections causing damage to trees that have economic value for growers (Fleischer et al. 1991); a lesser degree of uncertainty regarding appropriate application rates; and the relatively small size of these young prune trees, making direct foliar sprays feasible. We had reasonable confidence that direct foliar sprays of an RbCl solution to French prunes would result in substantial uptake of rubidium because French prunes are often potassium limited; foliar sprays of potassium are regularly used midseason on French prunes to supplement potassium; RbCl sprays would be done after fruit have matured, at which time there should be a particularly high potassium deficit; and leaves of French prune trees are active and engaged in nutrient uptake for an extended period during the fall (see Southwick et al. 1995).

Application of rubidium solutions to prune trees was timed to coincide as well as possible with the developmental period of the final generation of prune leafhoppers. Adults of this generation lay eggs in which *A. epos* overwinters, and we desired that these eggs have greatly enriched levels of rubidium. During 1991, foliar sprays were applied on 30 August, 12 September, and 23 September at each site. During 1992, foliar sprays were applied on 1, 15, and 29 September at the D20 site and 2, 16, and 30 September at the Chalone site. During 1993, foliar sprays were applied on 31 August, 10 and 20 September, and on 7 October. RbCl solution was applied to every other tree within a refuge in 1991; during 1992 it was applied to each tree in a refuge. In 1993, RbCl was applied to 50 French prune trees (2 rows of 25 trees) on the north edge of the orchard adjacent to the grape vineyard. A 5,000-ppm solution of Rb was applied twice to prune foliage on each spraying date. Based on experience with foliar potassium treatments, 2 sprays of 5,000 ppm should result in greater uptake than a single 10,000-ppm spray (Southwick et al. 1995). The solution was applied using a large (>350 L) herbicide sprayer with dual spray guns fitted with fine-spray nozzles. Trees were sprayed for a time period sufficient to obtain good coverage on all foliage, indicated by leaves beaded with drops of RbCl solution. Care was taken to spray all foliage on a tree, including the center and upper sections of the canopy. The time required to obtain the desired coverage was noted, and each tree was sprayed for the same amount of time to obtain equivalent coverage. For trees that were especially large or small, the time sprayed was adjusted up or down to obtain appropriate coverage for the particular tree. During 1991, 10.3 liters (across all 3 application dates) were sprayed on each tree for an average of 51.6 gm of Rb per tree at both sites. During 1992, 9.3 liters were sprayed on each tree at D20 for an average of 46.8 gm of Rb per tree. In total, 10.9 liters were sprayed on each tree at Chalone for an average of 54.7 gm of Rb per tree. During 1993, 10.5 liters were sprayed on each tree for an average of 52.6 gm of Rb per tree.

Sampling Foliage and Prune Leafhopper Adults and Eggs. At multiple times during and following the application of rubidium, foliage samples and adult prune leafhoppers were collected to assess uptake of rubidium. Foliage samples consisted of 5 leaves collected from each of 10 separate trees to which RbCl solution had been applied (total, 50 leaves). Adult prune leafhoppers were collected by aspirating individuals directly from leaves of trees to which RbCl solution had been applied. The number of specimens collected varied between samples because of differences in prune leafhopper density. In 1991 and 1993, foliage and prune leafhopper samples were collected on the same dates as (but before) RbCl was applied; additional samples were collected on 11 October 1991 and on 20 October and 20 November 1993. In 1992, samples were collected on 14 October and 5 November following the completion of rubidium sprays.

Samples were also collected from untreated trees for comparison. During 1991, every other tree at the Hayes and D20 sites was sprayed; control foliage samples were collected from the remaining, unsprayed, trees on the same dates as from sprayed trees. No control prune leafhopper specimens were collected in 1991. During 1992, all trees at the study sites were sprayed, therefore 2 large French prune orchards were chosen as unsprayed controls, south of Clements, CA (same as 1993 treatment site) and near Fairfield, CA. Foliage and prune leafhopper specimens were collected at these sites on 16 October and 17 November. All samples were held in a freezer before they were analyzed for rubidium content. Adult female prune leafhopper specimens collected on 5 No-

vember (treated sites) and 17 November (control sites) 1992 were dissected to obtain eggs for assessment of rubidium content.

In 1993, no control prune leafhopper specimens were collected. However, because only 50 trees in a large orchard were treated, additional prune leafhopper specimens were collected to assess the degree of mixing of adults between treated and untreated trees. Such mixing might tend to dilute, and therefore lower the effect of rubidium treatments on *A. epos* overwintering in treated trees. On 20 October, adult prune leafhoppers were collected from trees that were 1, 2, and 4 tree rows from treated trees, in addition to specimens collected directly from treated trees.

Naturally Occurring Rb Content in *A. epos*. *A. epos* was collected from vineyards that were distant from treated French prunes to determine the naturally occurring (endogenous) rubidium content. Specimens were collected by suspending 25.4 by 25.4 cm, yellow vinyl panels coated with petroleum jelly within the grape canopy. *A. epos* was collected from the following 3 sites: a vineyard block belonging to E. & J. Gallo Ranch near Livingston, CA, in June 1992; a vineyard east of Lodi, CA, in July 1993; and an experimental plot at the University of California Davis in July 1993. Panels were searched for *A. epos*, which were subsequently removed and rinsed in hexane to remove petroleum jelly. Rinsing was necessary to permit thorough chemical digestion of the specimens before rubidium was analyzed. Specimens were stored in a freezer for later analysis of rubidium content.

Sampling Overwintering *A. epos* in Prunes. To evaluate the success of *A. epos* labeling, individuals were collected at spring emergence directly from French prune trees that had been sprayed with rubidium the previous fall. Overwintering prune leafhopper eggs, in which *A. epos* overwinters, are oviposited underneath the bark primarily in 1- and 2-yr-old wood (Kido et al. 1984). The following 2 different methods were used to collect these overwintered *A. epos*: (1) twigs were pruned from treated trees, then placed in water in emergence containers (sealed containers with glass vials in which to capture emerging wasps) to collect emerging *A. epos*; and (2) twigs pruned from treated trees were examined for unemerged adult *A. epos*, which were subsequently dissected from the twig. We preferentially collected 1-yr-old twigs because they contain the majority of overwintering prune leafhopper eggs and because their smooth bark makes them easy to search for eggs. Twigs were collected from up to a height of 2 m; this may introduce some bias in our estimate of Rb content of *A. epos* to the extent that Rb labeling varies with height within a canopy.

During May 1992, 200 twigs were collected from sprayed trees at the Hayes and D20 sites and placed in emergence containers. From these twigs only 1 emerged *A. epos* was successfully recovered. Between 29 March and 7 May 1993, ≈250 twigs (between 20 and 50 cm in length) were collected from both study sites. From these twigs, 5 *A. epos* pupae were successfully removed by dissection and 2 adults were collected following emergence. On 7 May 1994, ≈200 twigs were collected at the Clements site. Roughly half of these were placed in emergence containers, yielding 18 individuals. The remaining twigs were examined for unemerged *A. epos*, yielding 51 individuals. All specimens were stored in a freezer for later analysis of rubidium content.

Retention of Rubidium by Labeled *A. epos*. To assess retention of rubidium by treated *A. epos*, individuals were reared on rubidium-treated grape vines. "Thompson seedless" grape vines were started from canes in February 1994. On 18 May, 10 healthy vines were selected from the initial set of canes and were subsequently treated with light foliar sprays of a 5,000-ppm RbCl solution on a weekly basis using a hand-held spray bottle; Rb applications continued through 11 July. On 18–20 July, 2nd–5th instar grape leafhoppers (*E. elegantula*) were collected from an experimental vineyard block on the University of California Davis campus. Twelve cylindrical, nylon–organdy sleeve cages (19 cm diameter, 30.5 cm long) were suspended on the 10 treated grape vines. Thirty to 40 nymphs were confined within each of the 12 cages. By the 1st wk of September, grape leafhoppers confined on treated vines had developed into adults and had begun to lay eggs. *A. epos* were then reared from grape leaves collected in the field, released within the sleeve cages, and allowed to oviposit in grape leafhopper eggs.

On 15 September, the sleeve cages were removed, and grape leaves were subsequently removed from the treated vines and examined for parasitized grape leafhopper eggs containing adult *A. epos* that were ready to emerge. Grape leaves were kept healthy by placing petioles in sealed plastic vials with water. Gelatin capsules were placed over *A. epos* that were close to emergence; capsules were examined every 12 h for emerged *A. epos*. Between 15 and 30 September, 34 adult *A. epos* were collected from treated grape leaves. Upon emergence, individuals were assigned to 1 of the following 2 treatment groups: (1) immediately frozen (at <12 h old); and (2) held for ≥48 h before they were frozen. Individuals in treatment (2) were provided a honey–water solution, which was replenished every 12 h. All *A. epos* were dead at 48–72 h following emergence. All specimens were stored in a freezer for later analysis of rubidium content.

Analysis of Samples for Rubidium Content. *Preparation of Leaf Samples.* All leaves were processed and analyzed individually for rubidium content. Individual leaves were thoroughly rinsed in deionized water before processing to remove any residue of RbCl on the leaf surface. Sample mass was reduced by cutting a small number of disks from each leaf using a paper hole puncher. The

Table 1. Rubidium content of prune leaves and adult prune leafhoppers from Rb-treated and untreated French prune trees in 1991

Date	Leaves (μg/gm)[a]				Prune leafhoppers (ng/insect)[b]	
	Untreated		Rb-treated		Rb-treated	
	Hayes	D-20	Hayes	D-20	Hayes	D-20
30 Aug.			39.4 ± 11.2		15.8 ± 3.7, 2	
12 Sept.	40.7 ± 11.9	35.1 ± 11.8	3,947.3 ± 1,451.0	3,945.9 ± 1,706.5	856.5 ± 788.7, 12	845.8 ± 654.8, 8
23 Sept.	40.6 ± 15.8	41.2 ± 17.6	10,412.0 ± 6,023.2	6,399.1 ± 3,480.2	1,149.7 ± 798.5, 8	1,000.9 ± 419.2, 12
11 Oct.	56.5 ± 21.5	48.2 ± 16.5	12,020.5 ± 4,391.3	8,689.8 ± 3,984.5	2,058.5 ± 726.4, 8	1,780.0 ± 346.2, 12

[a] Values are mean ± SD; *n* is 50 for all samples.
[b] Values are mean ± SD, *n*. Leaf and prune leafhopper samples were collected on same dates of, but before, Rb applications.

resulting sample was then dried at 30°C for at least 12 h and weighed to the nearest 1/10 mg. The typical mass analyzed was between 4.5 and 5.5 mg dry weight. Leaf samples were digested using the following 2-step wet-oxidation procedure: (1) place sample in 100 μL concentrated nitric acid and heat at 30°C for at least 12 h, (2) add 100 μL 30% hydrogen peroxide and heat at 30°C for at least 12 h. Samples were subsequently diluted to 400 μL.

Preparation of Adult Prune Leafhopper. All adult prune leafhoppers were digested using the following 2-step wet-oxidation procedure: (1) place specimen in 50 μL concentrated nitric acid and heat at 30°C for at least 12 h, (2) add 50 μL 30% hydrogen peroxide and heat at 30°C for at least 12 h. Samples were subsequently diluted to 200 μL.

Preparation of A. epos and Prune Leafhopper Eggs. Both *A. epos* adults and prune leafhopper eggs were digested using the same 2-step wet-oxidation procedure: (1) place specimen in 2 μL concentrated nitric acid and heat at 55°C for exactly 2 h, (2) add 3 μL 30% hydrogen peroxide and heat at 55°C for exactly 2 h. Samples were subsequently diluted to 50 μL. Specimens were not weighed before digestion because of their small mass.

Spectroscopy. Rubidium content of all samples was determined through flame emission spectroscopy using an Instrumentation Laboratories AAS/AES model 751. Emission at 780 nm (the wavelength of photons emitted by rubidium atoms in an excited state) was measured as an area integration across 4 s; thus, an entire sample was used in obtaining a reading. Rubidium content of specimens of the minute size of *A. epos* adults (<0.5 mm in length) and prune leafhopper eggs are typically obtained through atomic absorption spectroscopy using a graphite furnace due to its greater sensitivity (see for example Jackson et al. 1988). We were able to obtain readings for adult *A. epos* and prune leafhopper eggs using flame emission spectroscopy by taking the following 4 steps to increase measurement sensitivity: (1) installing a long-wavelength photomultiplier to increase sensitivity at 780 nm; (2) using high-purity acetylene gas to minimize contamination of the flame, thus reducing signal noise at long wavelengths; (3) maintaining low sample volume (50 μL for each specimen); and (4) aspirating the entire 50 μL sample during a single area-integration reading, thus maximizing the measurement obtained on each specimen.

Results and Discussion

Rubidium Content of French Prune Tree Foliage. Sprays of RbCl on French prune tree foliage resulted in substantial increases in rubidium content of foliage for all sites and years (Tables 1–3). Rubidium concentrations 2–3 wk after the final application were roughly 200 times the background level in 1991, 40 times the background in 1992, and 115 times the background in 1993.

Uptake of rubidium by prune foliage was greatest during the week after the 1st spray in 1991 and 1993 (see Tables 1 and 3). Later sprays resulted in proportionally smaller increases in rubidium con-

Table 2. Rubidium content of prune leafhoppers and adult prune leafhoppers from Rb-treated and control orchards in 1992

Date	Control		Rb-treated		
	Clements	Fairfield	Date	D-20	Chalone
			Leaves (μg/gm)[a]		
16 Oct.	110.8 ± 29.6	66.4 ± 17.2	14 Oct.	2,478.1 ± 1,118.4	4,035.7 ± 1,543.6
17 Nov.	38.1 ± 9.2	26.6 ± 5.9	5 Nov.	2,775.4 ± 1,540.8	4,674.7 ± 2,065.9
			Prune leafhoppers (ng/insect)[b]		
16 Oct.	1.3 ± 0.6, 21	1.4 ± 0.3, 20	14 Oct.	72.1 ± 46.7, 8	116.8 ± 66.2, 17

[a] Values are mean ± SD; *n* is 50 for all samples.
[b] Values are mean ± SD, *n*.

Table 3. Rubidium content of leaves and adult prune leafhoppers from Rb-treated orchard in 1993

Date	Leaves[a] (μg/gm)	Prune leafhopper[b] (ng/insect)
31 Aug.	72.1 ± 21.6	5.1 ± 1.7, 18
10 Sept.	4,592.5 ± 2,590.8	426.2 ± 189.3, 21
20 Sept.	5,416.6 ± 2,753.8	231.8 ± 128.5, 31
7 Oct.	7,265.1 ± 2,467.2	313.2 ± 172.6, 20
20 Oct.	8,407.5 ± 1,961.7	315.1 ± 146.9, 33
20 Nov.	5,738.9 ± 1,607.8	74.4 ± 59.3, 39

Leaf and prune leafhopper samples were collected before Rb applications on 31 August through 7 October.

[a] Values are mean ± SD; *n* is 50 for all samples.

[b] Values are mean ± SD, *n*.

Table 4. Rubidium content of eggs dissected from adult female prune leafhoppers from Rb-treated and control orchards in 1992

Site	*n*		ng/egg	
	♀♀	Eggs	Mean ± SD	Range
		Control		
Fairfield	4	10	0.24 ± 0.16	0.10–0.62
Clements	6	9	0.41 ± 0.31	0.10–0.77
		Rb-treated		
Chalone	7	24	15.02 ± 9.73	2.8–35.9
D-20	1	7	3.4 ± 2.28	0.60–6.77

tent. The latest sampling of prune foliage in a particular year was done in 1993 on 20 November, which was ≈6 wk after the final application of RbCl and 4 wk after the previous foliage sample was taken. Rubidium content of foliage decreased during this 4-wk period by 32% (Table 3). This decrease in rubidium content may be the result of mobilization of materials out of leaves before shedding. There was large variation in the maximum foliar rubidium concentrations achieved during the 3 yr. Most notably, the concentrations achieved in 1992 were 33% and 42% of the maximum levels achieved in 1991 and 1993, respectively. During 1992, prune leaves began to turn color and shed in late September, earlier than in 1991 or 1992. We suspect that these lower rubidium concentrations in 1992 may be the result of changes in leaf chemistry before shedding.

Our results demonstrate that foliar sprays are an effective means of achieving enrichment of rubidium in foliage of prune trees for purposes of elemental labeling studies. It is notable that substantial enrichment was achieved despite the fact that it was near the end of the growing season and prune leaves were fully mature. Thus it is not necessary for foliage to be in an active phase of growth to achieve rubidium enrichment through foliar sprays. The successful labeling of foliage likely was aided by the fact that French prunes have a high demand for potassium and that sprays were applied following fruit maturation, which is a period of particular potassium deficiency. Foliar sprays may represent an efficient means of elevating rubidium concentration in other tree systems when trees are relatively small and direct trunk injections are undesirable.

Rubidium Content of Prune Leafhopper Adults and Eggs. Enriched rubidium in French prune tree foliage resulted in substantial increases in rubidium content of adult prune leafhoppers for all sites and years (Tables 1–3). Rubidium content of prune leafhoppers 2–3 wk following the final application was roughly 130 times the background level in 1991, 65 times the background in 1992, and 60 times the background in '93.

The greatest proportional increase in rubidium content occurred during the week following the 1st spray in 1991 and 1993 (Tables 1 and 3). This suggests that most of the increase in rubidium content of adult prune leafhoppers was caused by short-term feeding rather than longer-term buildup in tissues during development. The rubidium content of prune leafhoppers varied between years and reflected differences in foliar concentrations of rubidium (Tables 1–3). In 1993, rubidium content of adult prune leafhoppers dropped dramatically at the final sampling on 20 November, apparently in response to the decrease in foliar concentration (Table 3).

Analysis of rubidium content in eggs dissected from adult female prune leafhoppers indicated that rubidium was transferred into eggs of prune leafhoppers at sprayed sites (Table 4). Eggs dissected from adult prune leafhoppers from treated sites had an average rubidium content 38.7 times greater than eggs from control sites. There was a large amount of variability in rubidium content of eggs from sprayed sites; nevertheless, all but 1 egg (from the single female collected at the D20 site) had a rubidium content >5 times the average for the unsprayed sites. Interestingly, there was nearly as much variability among eggs within females as there was in rubidium content of eggs from all females (SD of 9.7 versus 11.9, respectively; Chalone site, 1993). The implications of this result are not clear, but it may be the result of feeding on prune leaves with variable rubidium concentrations (see Tables 1–3).

Rubidium content of adult prune leafhoppers from treated and adjacent untreated trees in 1993 suggest that there was minimal mixing of adult prune leafhoppers between trees (Table 5). Based on a threshold of 10.4 ng Rb per insect (mean + 3 * SD for 31 August) (Table 3), all adult prune

Table 5. Elemental labeling of adult prune leafhoppers collected at increasing distances from Rb-treated French prune trees in 1993

Distance[a]	*n*	No. labeled (%)
0	34	34 (100)
1	39	9 (23)
2	51	5 (10)
4	49	3 (6)

[a] Distance is in tree-rows from Rb-treated trees.

Table 6. Rubidium content of *A. epos* from untreated vineyards (endogenous) and from Rb-treated French prune trees

Source			Rb content		
Site	Date	Method	*n*	ng Rb/insect ± SD	90% cutoff
			Endogenous		
Gallo	June 1992	Trap	75	0.110 ± 0.154	0.182
UCD	July 1993	Trap	130	0.020 ± 0.072	0.110
Lodi	July 1993	Trap	291	0.052 ± 0.095	0.107
Pooled			498	0.052 ± 0.105	0.129
			Rb-treated		
D-20	May 1992	Emrg	1	0.731 ± —	—
D-20	May 1993	Emrg	1	−0.082 ± —	—
Chalone	May 1993	Emrg	4	0.243 ± 0.179	0.487
Chalone	May 1993	Dsct	2	0.291 ± 0.258	0.474
Clements	May 1994	Emrg	18	0.231 ± 0.176	0.406
Clements	May 1994	Dsct	51	0.185 ± 0.210	0.474
Pooled			77	0.205 ± 0.208	0.487

Anagrus epos were obtained by 3 methods: trapping on sticky card (trap); allowing to emerge from collected prune twigs (emrg); dissecting out of prune leafhopper eggs layed in collected twigs (dsct). Ninety percent of sample falls at or below given cutoff.

leafhoppers from treated trees had substantially increased levels of rubidium. The proportion of labeled adult prune leafhoppers dropped to 23, 10, and 6% for trees that were 1, 2, and 4 rows away, respectively, from treated trees. This result suggests that there was negligible dillution of prune leafhopper labeling because of movement of adult prune leafhoppers into or out of the treated area. Therefore, the 50 trees treated at the Clements site in 1993 will produce labeling of *A. epos* comparable to a small, isolated stand of treated French prune trees.

Labeling of Overwintered *A. epos*. In total, 498 *A. epos* were collected from 3 separate control grape vineyard sites and analyzed for rubidium content (Table 6). An average of 0.052 ng Rb per individual was calculated as the endogenous rubidium content for *A. epos*. This is likely an underestimate of the true rubidium content because we were operating at the detection limit of the instrumentation and the rubidium content of some individuals was unreadable (yielding negative readings). Nevertheless, the measured endogenous level was comparable among the 3 sites and provides a solid basis for comparison against *A. epos* from treated prune trees.

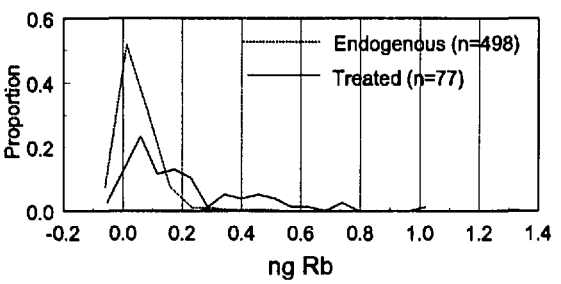

Fig. 1. Distribution of rubidium content of *A. epos* sampled from the endogenous population (n = 498) and from RbCl treated French prune trees (n = 77).

Treatment of French prune trees with RbCl in the fall resulted in increased rubidium content in *A. epos* emerging the following spring (Table 6). The average rubidium content of *A. epos* that overwintered in treated trees was 0.205 ng per individual, 3.9 times the average rubidium content of endogenous wasps. The mean and distribution of rubidium content for treated *A. epos* was comparable between 1993 and 1994 and between sampling methods (Table 6). There was a large degree of overlap in rubidium content between endogenous *A. epos* and those emerging from rubidium-treated trees (Fig. 1). Roughly 50% of the *A. epos* from treated trees had a rubidium content <1 SD above the endogenous average (0.157 ng Rb), making them essentially indistinguishable from the background. The low degree of labeling relative to that achieved in parasitoids in the laboratory (for example, Jackson et al. 1988) is not surprising given the following 4 required steps: (1) enrichment of rubidium in foliage in the fall, (2) enrichment of rubidium in adult prune leafhoppers and their eggs, (3) incorporation of rubidium by *A. epos* parasitizing late-season prune leafhopper eggs, and (4) retention of increased rubidium content by developing *A. epos* through the winter months until emergence in the spring. The degree of labeling was further complicated by the variability in rubidium content observed in prune leafhopper eggs both between and within females (Table 4). Although larger and more consistent increases in rubidium content of overwintering *A. epos* might be obtained through modifications to the RbCl treatment method (for example, more concentrated RbCl solutions, beginning applications sooner), the concentrations achieved in foliage and adult prune leafhoppers were high and approached those at which toxic effects begin to occur in other insects (VanSteenwyk 1991). The labeling of a small, overwintering parasitoid achieved in this study likely

represents a limit with respect to the ability to enrich rubidium content of small insects across time and across trophic levels.

The increase in rubidium content of *A. epos* emerging from treated trees (3.9 times the endogenous average) is not likely to result in adverse effects on behavior. Jackson et al. (1988) found that adult *Anaphes ovijentatus* Crosby & Leonard (=*iole* Girault) (Hymenoptera: Mymaridae) reared on rubidium-enriched hosts exhibited no adverse effects on longevity and fecundity when their average rubidium concentration was 5.5 times the endogenous level and exhibited minor effects when the average was 12.3 times the background. In similar studies with *Microplitis croceipes* Cresson (Hymenoptera: Braconidae), Hopper and Woolson (1991) found uncertain, minor adverse effects in adult wasps with an average rubidium concentration of >50 times the endogenous level.

Successful use of flame emission spectroscopy to measure rubidium content in this minute parasitoid has provided a logistical advantage over the use of a graphite furnace. Measurement of rubidium using a graphite furnace requires several minutes per sample compared with ≤15 s per sample using flame emission. This is a significant benefit in a study involving the analysis of hundreds of specimens from multiple sites and years. Use of flame emission spectroscopy provides the following 3 additional practical benefits: (1) decreased learning time, (2) greater dependability and consistency (especially if the use of an older instrument is required), and (3) lower expense of purchasing instrumentation designed solely for atomic emission.

Retention of Rubidium by Labeled *A. epos*. *A. epos* reared from grape leafhopper eggs on treated grape vines had increased rubidium content as compared with the endogenous level (Fig. 2). The mean rubidium content of individuals at emergence (<12 h old) was 1.23 (1.38, n = 16) ng Rb, 23 times the endogenous average of 0.052 ng. Rubidium content decreased to a mean of 0.53 (0.31, n = 18) ng in individuals between 48 and 72 h old, which had been provided a honey–water solution, representing an average decrease of 57%. Of particular interest is the fate of individuals that had levels of rubidium comparable with *A. epos* that emerged from treated prune trees; specifically, those with rubidium content of <1.0 ng and >0.2 ng Rb (see Fig. 1). At emergence (<12 h old), 50% of *A. epos* had a rubidium content between 0.2 and 1.0 ng, whereas 18% fell below 0.2 ng Rb. At 48 h following emergence, 80% still had a rubidium content within this range but only 10% fell below 0.2 ng Rb. Because no individual lived for >72 h, there was little degradation of the rubidium label over the adult life span for individuals that emerged with between 0.2 and 1.0 ng Rb. Because individuals were provided with honey–water solution, and most were observed feeding on the solution, this experiment should overestimate the availability of food to *A. epos* in the field and therefore should overestimate the rate of replacement of rubidium with potassium following emergence. This suggests that *A. epos* emerging from rubidium-treated trees with elevated levels of rubidium should retain the label throughout their adult life in the great majority of cases.

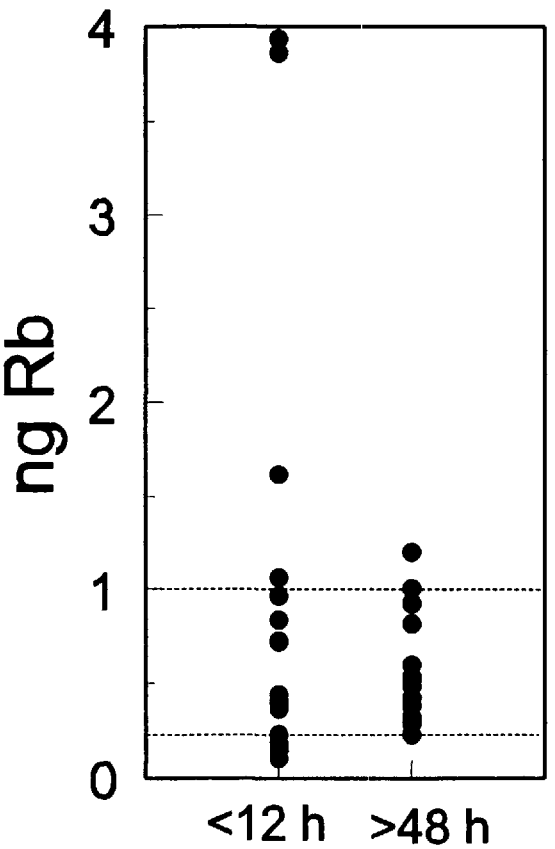

Fig. 2. Rubidium content of *A. epos* <12 and >48 h following emergence from rubidum enriched grape leafhopper eggs. Maximum observed lifespan was 72 h following emergence. Dashed lines bracket the range from 0.2 to 1.0 ng Rb per individual.

Potential for Study of Dispersal from Overwintering Refuges. Our results have demonstrated that a large portion of *A. epos* overwintering in French prune trees treated with RbCl will emerge in the spring with a detectable elemental label and will retain the label throughout their lifetime. Thus, it should be possible to monitor the dispersal of *A. epos* from a treated French prune tree refuge into an adjacent grape vineyard by collecting large numbers of *A. epos* within the vineyard early in the season and measuring their rubidium content individually. However, appropriate interpretation of the rubidium contents of captured *A. epos* will require modification of the conventional approach to rubidium labeling studies.

Conventionally, a captured insect is considered to be labeled if its rubidium content is >3 SD above the mean endogenous content (Stimman

1974, VanSteenwyk 1991). This results in a <0.001 probability of incorrectly classifying an insect as labeled, assuming the rubidium content of untreated individuals is normally distributed. It also results in a small probability of incorrectly classifying an individual as not treated (that is, not from treated vegetation) if rubidium applications result in greatly increased rubidium content such that there is little overlap with the endogenous distribution. This is commonly the case in laboratory studies and most field studies (Akey et al. 1991). Our results violate these assumptions in 2 ways. First, it is worth noting that neither the endogenous nor the rubidium-treated populations of *A. epos* have a normal distribution (Fig. 1). Second, rubidium treatments to refuges did not result in consistently large increases in rubidium content of emerging *A. epos;* rather, there is a large amount of overlap between the endogenous and treated populations (Fig. 1). Using the conventional approach for illustration purposes, we obtain a threshold of 0.367 ng Rb for considering an individual as labeled (see Table 6). Only 20% of the treated population is above this threshold; using this as a criterion for considering an individual as labeled would greatly underestimate the proportion of captured *A. epos* that originated in the refuge. Appropriate interpretation of the rubidium contents of captured *A. epos* will require a more sophisticated statistical approach that incorporates the information available in the probability distributions for endogenous and treated *A. epos* populations (Fig. 1). Such an approach would produce probabilistic rather than absolute statements concerning how many individuals belong to the treated group. Nevertheless, the distribution of rubidium content of *A. epos* emerging from treated prune trees is clearly distinct from that for the naturally occurring population, and appropriate analyses will yield valuable information concerning the dispersal of *A. epos* from French prune tree refuges.

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