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Labeling *Anaphes ovijentatus* (Hymenoptera: Mymaridae), an Egg Parasite of *Lygus* spp. (Hemiptera: Miridae), with Rubidium

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ABSTRACT The eggs of *Lygus hesperus* (Knight) were labeled with the element rubidium (Rb) by rearing the nymphs and maintaining the adults on diet with 100, 500, or 1,000 ppm rubidium chloride (RbCl). *Anaphes ovijentatus* (Crosby and Leonard), a parasite of *Lygus* spp. eggs, was marked with Rb concentrations above laboratory and field endogenous levels when reared from labeled eggs of *Lygus* adults fed diets with 500 and 1,000 ppm RbCl. Rb concentrations remained sufficiently high to distinguish labeled parasites from those collected in alfalfa fields for 4 d. The parasites that developed in eggs of *L. hesperus* reared on diet with 1,000 ppm RbCl tended to be shorter-lived and to produce fewer progeny than those from eggs of *Lygus* fed diets with lower concentrations, but only differences in longevity of males were statistically significant.

KEY WORDS Insecta, physiological ecology, biology, trace element

Anaphes ovijentatus (Crosby and Leonard) is a minute parasitic wasp, 0.5 mm in length, that develops in eggs of *Lygus* spp. and other mirids (Stoner & Surber 1969, Jackson & Graham 1983). It is a significant parasite of *Lygus* spp. on a number of host plants in the southwestern desert of the United States (Graham et al. 1986) and on alfalfa in Indiana (Sillings & Broersma 1974). A labeling technique would be useful to study movement and dispersal of the wasp and to distinguish released parasites from others indigenous to the area.

Any labeling technique must affect the biology and behavior of the insects, and the environment, minimally. Based on available literature and on preliminary tests, we selected for trial the element rubidium (Rb) as an internal physiological marker.

Since Berry et al. (1972) proposed this technique, several workers have labeled phytophagous insects by treating their host plants with a solution of rubidium chloride (RbCl) (Frazer & Raworth 1974; Stimmann 1974; Shepard & Waddill 1976; Graham et al. 1978a,b; van Steenwyk et al. 1978b; Alverson et al. 1980; Wolfenbarger et al. 1982; Fleischer et al. 1986). Graham et al. (1978a) demonstrated the movement of Rb through the food chain from plants to several species of predaceous insects. Payne & Wood (1984) successfully labeled a phytophagous larva and its ichneumonid parasite by injecting RbCl into the trunks of pecan trees. Insects also have been labeled by adding RbCl to artificial diets. Thus, adults of Lepidoptera (Stimmann et al. 1973, Graham & Wolfenbarger 1977, van Steenwyk et al. 1978a) and of Diptera (McLean et al. 1979, Burns et al. 1983) have been successfully labeled with little effect on their biology when moderate levels of RbCl were used. In addition, the braconid parasite of *Heliothis* spp. larvae, *Microplitis croceipes* (Cresson), was labeled by the addition of RbCl and three other elements to the artificial diet of its host (K. R. Hopper, personal communication).

Materials and Methods

Lygus hesperus (Knight) were reared from hatch through the nymphal stages, then maintained as adults on artificial diet (Debolt 1982) to which had been added 0, 100, 500, 1,000, or 5,000 ppm RbCl. The RbCl was dissolved in water and added to the diets; then the diets were packaged as described by Patana & Debolt (1985). Labeled *L. hesperus* females deposited eggs in packets like those used for diet but filled with Gelcarin (Marine Colloids, Springfield, N.J.). These eggs were exposed to the parasites and held for development and emergence of the parasite wasps. Analyses were done on individually labeled and unlabeled (control) *L. hesperus* eggs and newly emerged parasite wasps. Additionally, wasps from eggs of *Lygus* reared on diet with 500 ppm RbCl were held in cages and provided with water and honey. Each day for 6 d, 10 wasps were analyzed to determine how long the labeled wasps could be distinguished from the control wasps.

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Table 1. Rubidium content of *A. ovijentatus* from eggs of *L. hesperus* reared on diet with different levels of rubidium chloride

RbCl (ppm) in diet	n^a	Avg (SE) Rb (ng) per wasp
100	11	0.33 (0.04)a
500	12	1.88 (0.27)b
1,000	12	4.17 (0.29)c
Control	10	0.34 (0.07)a

Means followed by the same letter are not significantly different ($P = 0.05$; Duncan's [1955] multiple range test).

[a] The number of individuals that were analyzed; three subsamples were analyzed from each wasp.

Table 2. Fate of rubidium in *A. ovijentatus* adults over a 6-d period[a]

Day	n^b	Rb (ng) per whole wasp	95% CI
1	10	3.66	1.96–5.35
2	10	2.10	1.57–2.63
3	10	1.94	1.45–2.43
4	10	2.35	1.92–2.77
5	10	0.92	0.65–1.20
6	10	1.10	0.41–1.80

[a] The parasite adults were reared from eggs of *L. hesperus* fed a diet with 500 ppm RbCl.

[b] Three subsamples (each one-fifth of total) were analyzed for each of the 10 wasps.

Individual wasps were placed in a freezer (−10°C) within a few hours after emergence, held until all treatments were ready for analysis, then ashed in a muffle furnace at 650°C for 1 h. The ash was dissolved in 100 μl of 0.5 or 1.0% solution of nitric acid in distilled, deionized water. Fresh (<24 h old) *L. hesperus* eggs were digested in 1.0 μl of concentrated nitric acid at 60°C for 2 h in Reactitherm vials (SUPELCO, Bellefonte, Pa.), then diluted to 0.5% nitric acid with 199 μl of deionized water. Three aliquots of 20 μl each were analyzed for each specimen; thus each aliquot was 20% of the total sample for the wasps and 10% of the total for the *L. hesperus* eggs.

Rubidium was measured with a Perkin-Elmer (Perkin-Elmer Corporation, Norwalk, Conn.) model 5000 atomic absorption spectrophotometer with an HGA (Perkin-Elmer) graphite furnace assembly and programmer and a model AS-1 automatic sampler. An electrodeless discharge (EDL) lamp with a wavelength of 780.0 nm was used. Program parameters were 130°C for 20 s for drying, 700°C for 20 s for charring, and 2,300°C for 5 s for atomization. Standards of 0, 0.2, 0.4, and 1.0 ng of Rb in 20 μl 0.5% nitric acid were used according to the expected range of the samples. Standards were run with each set of samples and used to calculate regression equations to determine the actual quantities of Rb in each sample. Differences in levels of Rb in the wasps were tested for significance by analysis of variance (ANOVA) and where appropriate by Duncan's multiple range test (DMR) (Duncan 1955).

To determine the effects of the different *Lygus* diet levels on the parasites, newly emerged adults were sexed and paired, and each pair was put into a separate cage and held at 26°C, 70% RH and a 14:10 (L:D) photoperiod. The cages were made from 92.4-ml plastic vials with the bottom replaced with nylon organdy. A small oviposition packet (5 cm^2) containing 20–100 fresh *L. hesperus* eggs was placed in each cage and changed every 24 h. The larger numbers of eggs per packet were used for the first 4 d, when the parasites deposited most of their eggs (Jackson 1986). The wasps were observed daily and longevities were recorded. After exposure to the parasites, the egg packets were held in 100-ml plastic Petri dishes until emergence of the parasite progeny, which took 12–14 d. Packets were examined under a dissecting microscope and the number of parasitized eggs was determined by counting parasite pupae. This number was used as the count for the total number of progeny. Sex of the emerged parasites was also noted. Three replications of 8–10 pairs were done for each diet level, and differences in longevities and progeny production were tested for significance by ANOVA and DMR.

Voucher specimens of *Anaphes ovijentatus* have been deposited in the Canadian National Collection, Ottawa.

Results and Discussion

***Lygus* Eggs.** Few *L. hesperus* nymphs completed development to adults on the diet with 5,000 ppm RbCl; therefore, this level was not tested. *L. hesperus* fed diets with 100, 500, and 1,000 ppm RbCl produced eggs which contained significantly different amounts of Rb ($P < 0.01$, DMR), and eggs from all three levels had significantly greater amounts ($P < 0.01$) than occurred in the control eggs. The amounts in nanograms (and the standard errors) of Rb per individual egg were 0.25 (0.25), 4.80 (0.36), 17.69 (0.68), and 24.30 (1.04) for the control and the diet levels of 100, 500, and 1,000 ppm, respectively. The large standard error of the mean for the control was because one egg had a high level (2.50 ng) of Rb, but this level did not exceed the levels in any of the labeled eggs. With this one exception, the amounts of Rb in the *L. hesperus* eggs were less variable than were the amounts in the parasite adults.

***Anaphes ovijentatus* Adults.** Parasites reared from eggs of *L. hesperus* fed diet with 500 and 1,000 ppm RbCl contained significantly more ($P < 0.01$, DMR) Rb than those from the 0 or 100 ppm levels, which had similar amounts (Table 1). An average dry weight of an individual parasite (combined sexes) of 9.1 μg was determined by drying and weighing 26 wasps. The amount of Rb in the wasps averaged 0.02 and 0.05% of the total dry weight for those from the 500 and 1,000 ppm diet, respectively. The amounts of Rb in *L. hesperus* eggs averaged 0.08 and 0.11% of the total dry weight

Table 3. Longevity and fecundity of *A. ovijentatus* adults from eggs of *L. hesperus* reared on diets with different levels of rubidium chloride

RbCl level in diet (ppm)	Avg (SE) ♀[a]	Longevity (d) ♂[b]	No. progeny avg (SE)[a]
0	9.7 (1.79)	9.7 (1.20)a	72.3 (8.87)
100	7.7 (1.15)	7.6 (2.20)ab	68.0 (8.70)
500	9.8 (0.71)	8.0 (0.57)a	75.0 (4.51)
1,000	5.6 (1.49)	3.9 (0.38)b	54.9 (5.32)

[a] There were no significant differences between RbCl levels for female longevity or number of progeny ($P > 0.05$, ANOVA).

[b] Means followed by the same letter are not significantly different ($P = 0.05$; Duncan's [1955] multiple range test).

($\bar{x} = 22.1$ μg, $n = 8$) for the same respective diet levels.

Elimination of Rb. Parasites reared from eggs of *L. hesperus* fed 500 ppm RbCl lost 40–45% of the Rb by the second day after emergence but held this level through day 4. Levels dropped again for the fifth and sixth days, so that only 25–30% of the original amount of Rb remained (Table 2). Despite the drop in Rb, the levels in labeled wasps were consistently detectable ($P < 0.01$) from the background levels in laboratory controls (0.34 ± 0.07) for 4 d. Some levels were detectable on days 5 and 6, but there was an overlap in Rb quantities with some control wasps.

Anaphes ovijentatus were collected in alfalfa fields at Glover Ranch, located near Marana, Pima County, Ariz., and analyzed to determine the endogenous Rb levels. Rubidium levels in seven groups of wasps (71 total) averaged 0.69 ng Rb (±0.08). The 95% confidence interval for the field-collected wasps (0.63–0.74; df = 6; $t = 2.447$) overlapped with those from days 5 and 6, but not with days 1 to 4 (Table 2). Using the criteria of the average endogenous concentration plus three standard deviations ($\bar{x}$ + 3 SD) (Stimmann 1974), a wasp would be considered labeled if it exceeded 0.87 ng Rb. Approximately 50% of the wasps from days 5 and 6 contained amounts of Rb below this level.

Longevity and Progeny Production. The longevities of labeled and control wasps were highly variable (Table 3). Although wasps from eggs of *L. hesperus* fed on 1,000 ppm diet tended to be shorter-lived than those from controls or from the other treatments, the differences were not significant for females ($P > 0.05$). However, control males, and those from eggs of *L. hesperus* fed 500 ppm diet, lived significantly longer ($P < 0.05$, DMR) than those from the 1,000 ppm diet.

There were no overall significant differences in the number of progeny among the various treatments ($P > 0.05$; df = 3, 8; $F = 1.5583$) (Table 3). However, the wasps from eggs of *L. hesperus* reared on diet with 1,000 ppm RbCl generally produced fewer progeny than those wasps from the control or other treatments. Comparisons of the data from each of the treatments with the data from the control wasps also showed no significant differences. As expected, fecundity was extremely variable among individuals and among the replications. A greater proportion (61%) of the total oviposition occurred on the first day for those wasps from eggs of *L. hesperus* reared on diet with 1,000 ppm Rb than for wasps from the other treatments (39–48%). Otherwise, the ovipositional patterns were similar. The sex ratios of the progeny of wasps from the controls and from all treatments were similar. Sex ratios varied from 1.0:1.5 to 1.0:1.9 (males/females).

Using the element rubidium to label the minute parasitic wasps appears to be a workable method to study field movement and dispersal. The procedure of adding the rubidium salt, RbCl, to the host's diet is simple and, at the lower concentrations, has little effect on the parasites' longevity or progeny production. A concentration of 500 ppm labeled the wasps sufficiently well for 4 d to make them distinguishable from field-collected specimens.

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