

ELEMENTAL MARKERS FOR ENTOMOPHAGOUS INSECTS<sup>1</sup>

C. Glen Jackson

Honey Bee and Biological Control of Insects  
USDA, ARS, 2000 East Allen Road, Tucson, AZ 85719

## ABSTRACT

The use of rare elements to label entomophagous insects is a potentially valuable tool for studying survival and dispersal in the field. Prey insects have been labeled by treating their host plants with solutions of the chloride salts of the elements or by adding the elements to an artificial diet. At least three parasitoids, *Anaphes iole* (Girault), *Leiophron uniformis* (Gahan), and *Microplitis croceipes* (Cresson), and a predaceous hemipteran, *Geocoris punctipes* (Say), have been labeled by adding rubidium to artificial diets of their hosts or prey. The same procedures have been used to label *M. croceipes* with strontium, cesium, and dysprosium. The use of an atomic absorption spectrophotometer with a graphite furnace makes possible the analysis of micrograms of sample with the elements at levels of parts per billion per individual insect.

## INTRODUCTION

Information on dispersal, survival, and abundance of entomophagous insects is necessary for understanding the population dynamics of these beneficial insects and for assessing their potential effects on hosts or prey. Mark and recapture techniques provide a way to obtain this information. Such techniques are especially useful when the insects are indigenous and it is necessary to distinguish those released from others already present. Other papers in this supplement review the various methods of marking insects.

To be useful a label or mark must be durable and have minimal effect on the longevity and behavior of the insects (Southwood 1966). Additionally, it should be nonhazardous to the environment and easy to use and detect. Berry *et al.* (1972) proposed the use of rubidium (Rb) as a marker for native phytophagous insects, and subsequent studies indicate that elemental markers meet the desired criteria as listed by Southwood (1966). Although other elements can be used, most elemental labeling studies have employed Rb, which readily substitutes for potassium in plant and animal tissue. Burns *et al.* (1983) evaluated 10 elements as internal markers for the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) and concluded that Rb and strontium (Sr) were the best candidates. Phytophagous insects have been labeled

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by feeding on plants (including nectar) or on artificial diets containing the marker. Plants have been marked with Rb by spraying or injecting them (Stimmann 1974, Payne and Wood 1984), growing them in a solution (Frazer and Raworth 1974), or treating the seed prior to planting (Cheshire *et al.* 1987).

Elemental labeling of entomophagous insects involves first labeling the host or prey, which then becomes the source of labeled food for the parasitoid or predator. Thus, the effectiveness of the label and possible detrimental effects on both trophic levels must be considered. An artificial diet has been developed for rearing large numbers of the hemipteran predator, *Geocoris punctipes* (Say), eliminating the necessity of using prey insects (Cohen 1985). *In vitro* diets have been developed for several hymenopterous parasitoids of insect eggs and pupae and for two tachinid parasitoids of insect larvae (Greany 1986), but diets are not currently available for most parasitoids and predators. Since different stages of the host or prey insects serve as a food source for the entomophages, labeling methods must be effective for the proper stage(s). Many parasitic hymenopterans are very small and present difficulties in marking with paints, dyes, and powders. Elemental labeling provides a method of marking without having to handle the insects and without the possible environmental contamination of radioactive labels.

Graham *et al.* (1978) showed the movement of Rb through the food chain from plants sprayed with a rubidium chloride (RbCl) solution to phytophagous insects to predators. These included coccinellid larvae and adults; *Chrysoperla* spp., syrphids, *Geocoris* spp., *Nabis* spp., *Orius* spp. adults, and spiders. Some of the labeling of predators could have been due to their feeding on plant products such as nectar, plant juice, or pollen. Labeling of phytophagous insects by feeding on nectar of plants sprayed with RbCl has been demonstrated (Van Steenwyk *et al.* 1978, Hayes and Reed 1989). Payne and Wood (1984) injected pecan trees with RbCl and found that the foliage, the hickory shuckworm, *Cydia caryana* (Fitch), and a parasitoid of the hickory shuckworm, *Calliophialtes grapholithae* (Cresson), was labeled also. Other parasitoids and a predator have been labeled by adding RbCl or the chloride salts of other elements to an artificial diet for the hosts. Jackson *et al.* (1988) and Jackson and Debolt (1990) labeled the tiny mymarid wasp *Anaphes iole* (Girault) and the braconid *Leiophron uniformis* (Gahan), which are parasitoids of *Lygus* spp. eggs and nymphs, respectively. A braconid parasitoid, *Microplitis croceipes* (Cresson), of *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) larvae has been labeled with cesium (Cs), dysprosium (Dy), Rb, and Sr (Hopper and Woolson, in press). In this study Rb and Sr appeared to be the most useful, while Cs and Dy were difficult to detect with the analytical technique used. Concentrations of Cs and Dy in *M. croceipes* from hosts fed labeled diet overlapped with concentrations in parasitoids reared from hosts fed unlabeled diets. Cohen and Jackson (1989) used an artificial diet containing RbCl to label *G. punctipes*.

## TECHNIQUES

Individual *A. iole* and *L. uniformis* were frozen until ready for analysis, dried, then ashed in a muffle furnace for 1 hr at 650°C (Jackson *et al.* 1988, Jackson and Debolt 1990). The ash was dissolved in 100  $\mu$ l of 0.5 or 1.0% solution of concentrated nitric acid in distilled, deionized water. Further dilutions were necessary for *L. uniformis* to keep the sample concentrations within the range of the analyzer. Three aliquots of 20  $\mu$ l each were analyzed for each specimen. The same procedure was used for *G. punctipes* except that ashing required 2 hr at 650°C (Cohen and

Jackson 1989). Concentrated nitric acid was used to digest *M. croceipes* adults prior to analysis (Hopper and Woolson, in press).

For the wasps *A. iole* and *L. uniformis*, Rb was measured with a Perkin-Elmer model 5000 atomic absorption spectrophotometer with a HGA graphite furnace and programmer and a model AS-1 automatic sampler (Jackson *et al.* 1988, Jackson and Debolt 1990). An Electrodeless Discharge Lamp with a wavelength of 780.0 nm was used. Program parameters were 130°C for 20 sec for drying, 700°C for 20 sec for charring, and 2300°C for 5 sec for atomization. Standards were used according to the expected range of the samples and run with each set of samples. Similar procedures were used for the predator *G. punctipes*, except that the samples were dried at 100°C for 35 sec (Cohen and Jackson 1989). An atomic absorption spectrophotometer (Perkin-Elmer 3030) with an HGA 400 graphite furnace and AS-40 autosampler was used for *M. croceipes* (Hopper and Woolson, in press). Single element, hollow cathode lamps were used for Dy and Sr; electrodeless discharge lamps were used for Cs and Rb. Char and atomization times and temperatures were adjusted according to the element being tested. Pyrolytically-coated graphite tubes were used for all four elements.

### LABELING RESULTS

Levels of RbCl in the diets of the hosts or prey of entomophagous insects have ranged from 100 to 1428 ppm (70-1000 ppm Rb) (Table 1). Host diets containing 100, 200, 500, and 1000 ppm RbCl were sufficient to label adult *L. uniformis* above the controls (unlabeled), when they were fed to the host nymphs (Jackson and Debolt 1990). When *Lygus hesperus* Knight were reared through the nymphal stages, then maintained as adults, on diet with 100, 500, and 1000 ppm RbCl, their eggs contained significantly more Rb than did the eggs from *L. hesperus* fed diets without Rb. *Anaphes iole* that emerged from eggs of *L. hesperus* fed diet with 500 and 1000 ppm RbCl contained levels above that found in unlabeled wasps (Jackson *et al.* 1988).

TABLE 1. Mean Rubidium Concentration (PPM Dry Weight) in Three Species of Parasitoids and a Predator Reared From Rb-Labeled Hosts.

Species	Dry Wt. (mg)	Conc. of RbCl in Host Diet (ppm)				
		0	100	200(245 <sup>a</sup> )	500(392 <sup>a</sup> )	1000
<i>Anaphes iole</i>	0.0091	37	36	---	200	500
<i>Leiophron uniformis</i>	0.23	10	35	65	196	370
<i>Calliephialtes grapholithae</i>	2.33	110	--	399	1069	---
<i>Geocoris punctipes</i>	---	13	175	---	327	440

<sup>a</sup> Concentration of RbCl (ppm) in pecan shuck tissue, which served as a food source for the host of *C. grapholithae* (Payne and Wood 1984).

At the levels of RbCl (100-1428 ppm) in the host diet needed to label entomophagous insects, rubidium appears to have few adverse biological effects. However, there are some differences in the tolerance of the different species. At 1,000 ppm there were some significant differences in the effects of the four elements on the development time and adult longevity of *M. croceipes* (Hopper and Woolson, in press). However, the authors attributed these differences to uncontrolled variables rather than to the effects of the elements. There was a trend for decreased longevity and fecundity in *A. iole* with increased concentrations of RbCl (100-1000 ppm), but only male longevity was reduced significantly at the higher level (Jackson *et al.* 1988). The proportion of female progeny for *L. uniformis* reared from *L. hesperus* nymphs fed diet with 500-1000 ppm RbCl was reduced by 21-38% over the proportion from unlabeled wasps (Jackson and Debolt 1990). Development times for 4th and 5th instar *G. punctipes* were not affected by dietary rates of 100-1000 ppm RbCl (Cohen and Jackson 1989).

In insects labeled with Rb there is a general trend for a rapid loss of the element during the first few days after removal from a Rb-enriched diet, followed by a much slower decline afterward. A similar trend was found to occur in parasitoids and predators. The parasitoids *A. iole* and *L. uniformis* lost 40-45% and 66%, respectively, of the accumulated Rb within the first 24 to 48 hr after emergence from their host. Then the rates of loss decreased so that labeled *A. iole* could be distinguished from laboratory controls for 4 days (Jackson *et al.* 1988) and *L. uniformis* for 10 days (Jackson and Debolt 1990). The predator, *G. punctipes*, after removal from a diet with 500 ppm RbCl, lost 70% of the Rb by the end of 1 wk; but it was still distinguishable from unlabeled bugs (Cohen and Jackson 1989). Levels of Rb and Sr declined at an exponential rate in *M. croceipes* but were still distinguishable from levels in unlabeled, field-collected wasps for at least 20 days (Hopper and Woolson, in press).

## CONCLUSIONS

The element Rb has been used to label at least three parasitic Hymenoptera and a predaceous hemipteran by the simple process of adding RbCl to the diet of the host. The work of Graham *et al.* (1978) has shown that this technique may be useful for other predaceous arthropods, and the studies reviewed here suggest the application of this method for use with other parasitoids. The parasitoids, which have been studied, have hosts from Hemiptera (eggs and nymphs) and Lepidoptera (larvae). Other phytophagous insects from Coleoptera (larvae and adults), Diptera (larvae, pupae, and adults), Homoptera (nymphs and adults), and Lepidoptera (eggs, larvae, pupae, and adults) have been labeled, so this method should be useful for a wide range of parasitoids.

Other elements, such as Sr, Cs and Dy may also hold promise for labeling of entomophagous insects. The use of two or more elements would allow for double or multiple marking, but the effect of multiple labels on the biology and behavior of the parasitoids or predators would have to be tested. It is possible that any detrimental effects of two or more elements could be additive. Tobacco budworms, *Heliothis virescens* (F.), triply labeled with Rb-Sr-Cs showed reduced pupation and moth eclosion rates and an altered, female-biased sex ratio (Hayes 1989). There were similar effects in sex ratio and eclosion with Dy alone and in eclosion rates with Rb-Sr and Sr-Cs combinations. However, no apparent adverse effects on developmental time or longevity were found when *M. croceipes* were double or triple-

marked with Cs, Rb, and Sr combinations with each element at 1000 ppm in host diet (Hopper and Woolson, in press).

The rate of Rb loss from labeled insects is an important consideration for field studies on survival and dispersal. The rapid early decline in Rb levels after removal from an enriched food source may be due to excretion of food material from the gut. Later declines are influenced by feeding and metabolism rates and the concentration of electrolytes in the diet. Fairbanks and Burch (1968) found that higher levels of KCl or RbCl in the diet increased the rate of elimination of Rb in *Drosophila* spp. and *Megaselia scalaris* (Loew). This indicates that entomophagous insects feeding on a source high in potassium after being marked and released in the field would more rapidly lose a Rb label.

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