USING RUBIDIUM TO MARK A PREDATOR, *GEOCORIS PUNCTIPES* (HEMIPTERA: LYGAEIDAE)[1]

Allen C. Cohen and C. Glen Jackson
USDA, Agricultural Research Service
Biological Control of Insects Laboratory
Tuscon, AZ 85719
(Accepted for publication 1 June 1988)

ABSTRACT

Geocoris punctipes (Say) was fed artificial diet containing 0, 70, 350 or 700 ppm Rb from 4th-instar nymphal stage to adult stage. Adult weights and duration of nymphal stadia were not affected by Rb treatment. Adult survival was reduced at 350 ppm but was not significantly lower at 700 ppm than at 0 or 70 ppm Rb. Fifth-instar nymphs that were fed 350 ppm and kept under laboratory conditions (i.e., *ad libitum* food and water, 27°C, 40% RH) retained enough Rb for reliable detection 1 week after return to unlabeled diet.

Key Words: Predation, rubidium, marking insects, *Geocoris punctipes, Hemiptera, Lygae idae.*

J. Entomol. Sci. 24(1): 57-61 (January 1989)

INTRODUCTION

Since many programs of insect study require the labeling of specific individuals or groups, insect marking techniques have received a great deal of attention in the last 2 decades (e.g., Graham et al. 1978; Stimmann et al. 1973; and Van Steenwyk et al. 1978). Rubidium (Rb), being relatively non-toxic at a ppm concentration in insect diets while being detectable at ppb concentrations, has been widely used as a marking agent for insects. It is easy to administer and safe (compared to radioisotopes). Rb is presumed to replace K in biological systems (Fairbanks and Burch 1968).

Most studies of Rb as a marker for insects have been confined to the first and second trophic levels. An exception is the study of Payne and Wood (1984), who found that Rb was transferred from a phytophagous host to its ichneumonid parasite. Also, Graham et al. (1978) showed that Rb sprayed on plants as RbCl solution is transferred through the 2 successive trophic levels.

We needed a method for marking laboratory-reared and artificial diet-fed predators that were to be released in the field and recaptured. We undertook the present study on *Geocoris punctipes* (Say), to determine whether this predator could be labeled through its artificial diet; what quantities of Rb are required to safely mark individuals; and how persistent the marker is in this species. We utilized an artificial diet that has supported a *G. punctipes* culture for over 3 years as a convenient vehicle for directly administering the Rb marker.

[1] Mention of a proprietary product or trademark does not constitute endorsement or recommendation by USDA-ARS for its use over any other product.

MATERIALS AND METHODS

Geocoris punctipes used in this study were reared in laboratory culture for ca. 18 generations (ca. 3 years) on artificial diet (Cohen 1985). They originated from samples collected from an alfalfa field in the Avra Valley ca. 25 km northwest of Tucson, Az.

Third-instar nymphs were individually caged with diet packages (Cohen 1985), and water was provided via a sponge-stoppered vial. As these nymphs reached 4th-instars, they were placed individually in 22-ml plastic condiment cups with water in sponge-stoppered vials and provided with either test or control diet.

The diet used was the artificial diet described by Cohen (1985). Rubidium chloride (RbCl) when added was dissolved in the sucrose solution to yield final RbCl concentrations of 0 (for the control), 10, 50 or 100 mg/100 g diet (i.e., 0, 100, 500 or 1000 ppm RbCl or 70, 350 or 700 ppm Rb). Hereafter we will specify ppm Rb. All diets were provided in stretched Parafilm® casing formed into cylinders ca. 1 cm dia and 5 cm long (Cohen and Urias 1986). Because no antibiotics were added to these diets, food was replaced every other day.

Fresh body weight of newly eclosed 4th-instar nymphs and of the newly emerged adults were calculated by placing unanesthetized insects in gelatin capsules and weighing them to the nearest .01 mg on a Cahn 25® Microbalance. Cages with individual insects were examined daily to determine duration of each instar. Percent survival to the adult stage was also noted for each treatment.

In a separate test, 5th-instar nymphs were fed 350 ppm diet and kept under laboratory conditions (27°C, 40% RH) until they became adults, then transferred to 0 ppm diet. We selected the 350 ppm diet for this part of the experiment as an effort to circumvent undetected Rb toxicity at the higher (700 ppm) concentration. Because 700 ppm was safe to the insects, 350 ppm was considered safer. Rubidium concentrations were measured in adults at 1, 2, or 3 weeks after removal from the diets containing Rb to determine how long a detectable level of Rb was retained in the insects. Five replications of 10 insects each were done for each test. *Geocoris* nymphs fed unmarked diet were used as controls in all tests to establish background levels of Rb. Concentration of Rb in unlabeled diet was below limits of detection in the quantities of diet used in this study.

Individual adult insects to be analyzed for Rb content were dried, weighed, and ashed in a muffle furnace at 650°C for 2 h. The residues were dissolved in 100 µl of 1% HNO_3 in distilled H_2O. Aliquots of the solution (20 µl) were analyzed with a Perkin-Elmer® 5000 atomic absorption spectrophotometer equipped with an HGA graphite furnace and an EDL lamp with a wavelength of 780.0 nm. Each sample was dried at 100°C for 35 s, charred at 700°C for 20 s, ashed at 2300°C for 5 s with a 2500°C clean out for 3 s. Ramp times were 8 s, 10 s, 0 s and 1 s for each step, respectively.

Data were analyzed by an SNK Test (Sokal and Rohlf 1969) at $P = 0.05$ level.

RESULTS AND DISCUSSION

Developmental times (Table 1) were not significantly affected by Rb treatment at any concentration tested. Likewise, adult dry weights (both for males and females) were unaffected by Rb treatment (Table 1). However, the % survival to the adult stage was significantly lower at 350 ppm than at 0 or 70 ppm Rb. The

86% survival rate at 0 ppm Rb agrees with rates reported by Cohen and Urias (1986) for unlabeled diets.

The Rb content (Table 2) of *G. punctipes* averaged between 175.0 ppm (dry weight) and 440.5 ppm at dietary concentrations of ca. 3 ppm to 33 ppm diet dry weight. For a 10X increase of Rb in the diet there was a 2.5X increase in the Rb concentration in *G. punctipes.* Background Rb concentrations measured in *G. punctipes* fed unlabeled diet averaged 13.6 ppm (± 0.43 S.E.).

Table 1. Results of bioassays of *Geocoris punctipes* provided with diets (4th-instar through adults) containing the specified concentrations of Rb.

Treatment (ppm Rb in wet wt. of diet)	0	70	350	700
Duration of 4th-instar (days)	5.7 a	5.3 a	5.4 a	6.0 a
Duration of 5th-instar (days)	7.5 a	7.3 a	7.5 a	7.9 a
Adult wet weights (♀♀) (mg)	3.4 a	3.4 a	3.2 a	3.2 a
Adult wet weights (♂♂) (mg)	2.4 a	2.3 a	2.4 a	2.3 a
% Survival to Adult Stage*	86 a	81 a	53 b	71 ab

*Means converted by arcsin transformation and seperated by SNK; means in a row that are followed by the same letter are not significantly (P = 0.05) different. Each treatment included 5 replications with 10 individuals per replication.

Table 2. Means (± SE) of rubidium concentrations (ppm dry wt.) in carcasses of newly enclosed *Geocoris punctipes* adults fed diet with different amounts of Rb.*

	Diet treatment (ppm Rb in wet wt. of diet)			
	0	70	350	700
Rb concentration in insects	13.6 d (2.87)	175.0 c (23.54)	327.4 b (29.67)	440.5 a (24.79)

*Means separated by SNK; different letters beside means represent significant differences (P = 0.05). Tests involved 5 replications with 10 individuals per replication.

More than 28% of the average Rb concentration remained after 1 week, but only about 7.4% of the original level remained 2 weeks after discontinuation of Rb-feeding (Table 3). Residual Rb concentrations in carcasses after 1 week were consistently higher than in the control group. Neither of the 2-week nor 3-week treatments were significantly different from controls.

Table 3. Mean (± SE) rubidium concentrations (ppm/insect dry weight) of *Geocoris punctipes* adults removed from Rb-containing diet (500 ppm RbCl) at weekly intervals.*

	0 Weeks	1 Week	2 Weeks	3 Weeks
Treatment†	337.0 a	94.2 b	24.8 c	16.6 c
Concentration	(26.48)	(16.23)	(5.59)	(1.35)

* Means separated by SNK; different letters beside means represent significant differences (P = 0.05). These tests were started with ten 5th-instar nymphs, with 5 replications.

† Number of weeks after removal from Rb-containing diet.

Rubidium is a biologically rare element, which replaces potassium in plants and animals. Fairbanks and Burch (1968) found that ^{86}Rb was eliminated progressively faster with increasing amounts of either RbCl or KCl in the diet of *Drosophila* spp. or *Megaselia* sp. However, investigators who used high enough concentrations (e.g., Fleischer et al. 1986; Stimmann et al. 1973), Graham and Wolfenbarger 1977; Van Steenwyk et al. 1978) found that this replacement of K by Rb has biological limits.

We determined that rates of development were unaffected by various rates of Rb tested. However, at 700 ppm there were marginal, but not significant ($P > .05$), differences in % survival to the adult stage, and at 350 ppm there were significantly fewer adult emergences. The depression in survival at the middle level of Rb appears anomalous and remains unexplained. This rate anomaly is especially interesting and perplexing since it is the reverse of what Graham and Wolfenbarger (1977) found with respect to pupal weights and fecundity in the tobacco budworm, *Heliothis virescens* (Fabricius), fed diets containing 3 levels of Rb which are similar to those in the present study. In Graham and Wolfenbarger's study, the middle level of Rb actually appeared to benefit the insects. We decided to use the 350 ppm rate for this study and for future studies involving field releases because it was the lowest level tested that adequately marked insect for 1 week. We selected 1-week, post-release time as the minimum period to measure survival of our laboratory-reared insects in the field.

The present study demonstrated that *G. punctipes* could be marked with Rb by feeding 5th-instar nymphs diets containing RbCl at a concentration of about 350 ppm. This provides a marker that is clearly detectable for about 1 week after removal of the subjects from Rb enriched diet. This 1-week period of marker detectability is under laboratory conditions with insects kept at 27°C, 40% RH and with *ad libitum* food and water. The uncertainty of how the Rb marker will be affected by field conditions mandates field tests to determine the stability of the Rb marker in predators. However, it is evident from this study that laboratory-reared predators such as *G. punctipes* can be marked safely with RbCl and detected for at least a few days after release. This should be a valuable tool in the measurement of dispersal and in evaluation of laboratory-reared predators that are to be used in biological control programs.

ACKNOWLEDGMENTS

We thank Drs. Dennis Dunbar, Juan Lopez, David Byrne, and Robert Crocker for helpful reviews of an earlier version of this manuscript and Gene Neeman and Charmaine Verdugo for valuable technical assistance. We also thank Dr. Howard Morton from the Arid Land Watershed Management Research Unit for use of the atomic absorption spectrophotometer.

LITERATURE CITED

Cohen, A. C. 1985. Simple method for rearing the insect predator *Geocoris punctipes* (Heteroptera: Lygaeidae) on a meat diet. J. Econ. Entomol. 78: 1173-75.

Cohen, A. C., and N. M. Urias. 1986. Meat-based artificial diets for *Geocoris punctipes* (Say). Southwest. Entomol. 11: 171-76.

Fairbanks, L. D., and G. E. Burch. 1968. Whole body turnover of potassium traced by radiorubidium in adult *Drosophila* (Drosophilidae) and *Megaselia* (Phoridae). J. Insect Physiol. 14: 819-30.

Fleischer, S. J., M. J. Gaylor, N. V. Hue, and L. C. Graham. 1986. Uptake and elimination of rubidium, a physiological marker, in adult *Lygus lineolaris* (Hemiptera: Miridae). Ann. Entomol. Soc. Am. 79: 19-25.

Graham, H. M., and D. A. Wolfenbarger. 1977. Tobacco budworms: Labeling with rubidium in the laboratory. J. Econ. Entomol. 70: 800-2.

Graham, H. M., D. A. Wolfenbarger, and J. B. Nosky. 1978. Labeling plants and their insect fauna with rubidium. Environ. Entomol. 7: 379-83.

Payne, J. A., and B. W. Wood. 1984. Rubidium as a marking agent for the hickory shuckworm, *Cydia caryana* (Lepidoptera: Tortricidae). Environ. Entomol. 13: 1519-21.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman, San Francisco. 776 pp.

Stimmann, M. W., W. W. Wolf, and W. L. Berry. 1973. Cabbage loopers: biological effects of rubidium in the larval diet. J. Econ. Entomol. 66: 324-26.

Van Steenwyk, R. A., G. R. Ballmer, A. L. Page, and H. T. Reynolds. 1978. Marking pink bollworm with rubidium. Ann. Entomol. Soc. Am. 71: 81-84.