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RUBIDIUM AS A MARKER FOR MEXICAN BEAN BEETLES, *EPILACHNA VARIVESTIS* (COLEOPTERA: COCCINELLIDAE)[1]

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Abstract

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Mexican bean beetles, *Epilachna varivestis* Mulsant, were marked with rubidium by allowing larvae and adults to feed on foliage of bean plants treated with a rubidium chloride solution. Detection of rubidium was effected by atomic absorption spectroscopy. Adults which had fed for 4 days on rubidium-chloride-treated plants then transferred to untreated plants contained elevated levels of rubidium up to 34 days. The presence of rubidium was not detectable in the progeny of treated beetles at levels significantly different from controls. Consumption of rubidium-chloride-treated foliage produced no observable detrimental effects on the beetles. The technique allowed them to acquire a relatively permanent label through their normal feeding activity.

Introduction

Marking phytophagous insects with rubidium (Rb) was proposed by Berry *et al.* (1972) and has been shown to successively mark native populations of cabbage looper, *Trichoplusia ni* (Hübner), and the imported cabbageworm, *Pieris rapae* (L.) (Stimmann *et al.* 1973; Stimmann 1974). Rubidium is similar in its chemical properties to potassium but is found in very small amounts in natural systems. It can be applied to plants without causing phytotoxicity or toxicity to the phytophagous insects feeding on treated foliage, and up to 10,000 ppm of Rb had little effect on cabbage looper development, longevity, mating, fecundity, fertility, or response to a synthetic pheromone by adult moths (Stimmann 1974). Plants readily take up this element via foliar sprays or through the soil and translocate it throughout their system (Levi 1970). Phytophagous insects which feed on these plants are marked with elevated levels of Rb which are easily detected by atomic spectrophotometry.

There are several distinct advantages to using Rb as a marker for studying insect movements: migration, dispersal, etc. This method obviates the need for satisfying the prerequisites of most mark–release–recapture techniques as described by Southwood (1969). In addition, the possibility of contamination by radio-isotopes is eliminated and insects to be studied can be marked without disturbing them in their natural habitat. The technique lends itself especially to studies on movements of insects within and between fields. It probably would not have a significant advantage over other techniques which require capture and release of individuals.

Knowledge of the movements of Mexican bean beetles, *Epilachna varivestis* Mulsant, may be essential to the development of effective control tactics for this species which can be a major defoliator of snap beans, *Phaseolus* spp., and soybeans, *Glycine max* (L.). Studies of insect population dynamics and development of computer simulation models should also include immigration and emigration. Movements of Mexican bean beetles from overwintering sites to snap beans occur in early spring and later in the season these beetles and(or) their progeny move to soybeans. Their movements within or between soybean fields may be dictated by the availability of food or cultivar preference by the beetles.

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This study was designed to observe the practical feasibility of using Rb as a marker for Mexican bean beetles by application of RbCl solutions to snap beans.

Methods and Materials

Experiments were conducted using potted bean plants (*Phaseolus* sp.), 20–26 cm tall, which were grown inside a greenhouse. Endogenous levels of RbCl were determined for all larval stages, pupae, and adults of the Mexican bean beetle and also for leaves of the bean plants. In addition, a total of 215 adults were collected from untreated soybean (*Glycine max* (L.) Merrill) fields and analyzed for Rb.

Plants were treated until run-off with a RbCl solution at a rate of approximately 1100 g per hectare using a 7.6 l. hand sprayer. After the plants were allowed to dry, Mexican bean beetles were introduced onto them. Immediately after drying, leaves from the treated plants were also analyzed for Rb. The RbCl was obtained from Kawecki Berylco Industries, Inc., Box 1A, Revere, Pa. 18953.

Mexican bean beetle adults were allowed to feed on the treated plants for 4 days, then removed to untreated foliage or checked for Rb with subsequent samples analyzed at the end of 4, 8, 12, 16, 24, and 34 days. Samples of 20 beetles were analyzed at each time interval except for day 34, then 13 beetles were analyzed. Third- ($n = 23$) and fourth-stage ($n = 29$) larvae were also placed on treated plants, allowed to develop to adults and levels of Rb determined. Eggs laid by adults which had fed for 4 days on treated foliage were allowed to hatch and develop on untreated plants. The resulting adults were analyzed for Rb.

Because first- and second-instar larvae were too small to allow detection of Rb in individual animals, these samples were pooled with 30 first and 16 second instars per sample. Other insects to be analyzed were placed individually in vials and ashed in a furnace at 600°C for 3 h. The ashed samples were then dissolved in a 5-ml solution of 0.25 *N* HCl and stirred with a Vortex mixer. Rubidium levels were measured for each sample by atomic absorption spectroscopy operating in the flame emission mode at 780.02-nm line for Rb.

Results and Discussion

Mean endogenous level of Rb in leaves of snap beans was 69 μg/g of dry weight. An increase to 3700 μg/g was observed on leaves after treatment to run-off with RbCl solution.

Naturally occurring levels of Rb in all larval stages and adults of Mexican bean beetles are presented in Table I. The amounts of Rb in fourth instars through the adult stage are essentially the same. Highest levels were detected in fourth instars where a mean (±S.D.) level was 0.19 (±0.04) μg/insect. Endogenous levels of Rb in adult beetles collected from soybean fields were not significantly different from those from beetles reared in the greenhouse.

Table I. Endogenous levels of rubidium in stages of the Mexican bean beetle, *E. varivestis*

Stage	μg/insect	Range
1st instars	0.002	–*
2nd instars	0.012	–*
3rd instars	0.027	0.023–0.030
4th instars	0.190	0.080–0.260
Pupae	0.100	0.070–0.140
Adults	0.140	0.090–0.180

*Levels of rubidium in individual 1st and 2nd instars were too low to allow detection, thus samples were pooled.

Table II. Levels of rubidium in adult Mexican bean beetles, *E. varivestis,* after feeding for 4 days on RbCl-treated bean plants then transferred to untreated plants

Days post-treatment	*N*	Mean ± S.D. (μg/beetle)	Range
0	20	6.30±3.23	3.0 –15.0
4	20	1.43±1.89	0.30– 3.5
8	20	0.67±0.86	0.20– 3.0
12	20	0.56±0.50	0.22– 2.3
16	20	0.48±0.23	0.20– 1.0
24	20	0.21±0.23	0.05– 1.1
34	13	0.69±0.55	0.24– 2.30

Larvae which were placed on Rb-treated plants as third instars contained an average (±S.D.) of 2.74 (±1.19) μg/insect upon reaching adulthood and levels of Rb in adults which had developed on treated plants from fourth instars contained 5.71 (±1.74) μg/insect. These elevated levels of Rb produced no observable effects on beetle growth, development, and behavior.

When adult beetles were allowed to feed on bean plants treated with Rb, an average of 6.3 μg/insect was recorded 4 days later (Table II). A rapid decline in the amount of Rb present in adults after 4 days, and a subsequent leveling off in the quantity of Rb after 8 days, suggest that much of the material which was detected initially was probably present in foliage in the alimentary canal of the insect. Detectable amounts of Rb were present throughout the 34-day period which indicated that the element was probably incorporated into the tissue and may have replaced some potassium (Evans and Sorger 1966). Amounts of Rb significantly higher than controls were found in 93% of the beetles which were allowed to feed on treated plants for 4 days and removed to untreated plants for 34 days. Thus, the use of Rb should provide a relatively permanent marker after only a brief period of feeding by larvae and adults on Rb-treated plants.

Adult beetles (n = 20) from eggs laid by beetles which had fed for 4 days on treated plants contained levels of Rb which were not significantly different from the control group. Thus, our studies revealed no carry-over of the element to subsequent generations.

These findings demonstrate the practical feasibility of using Rb as a marker for the Mexican bean beetle. Because of the movements of beetles from snap beans to soybeans with later dispersal between soybean fields, this element provides a basis for a rapid, yet inexpensive method for marking large numbers of Mexican bean beetles in the field.

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