Comparison of Foliar and Soil-Drench Applications of Aqueous Rubidium Chloride Solutions to Plants for Marking Feeding Aphids (Homoptera: Aphidae)[1]

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ABSTRACT Aqueous solutions of rubidium chloride, RbCl, (10 g/l, 10,000 ppm) were applied to foliage or soil of potted bell pepper and tomato. After 48 h, green peach aphid (*Myzus persicae* Sulzer) and potato aphid (*Macrosiphum euphorbiae* Thomas) were transferred to pepper and tomato, respectively. Leaf and aphid samples were collected after 6 d; remaining aphids were transferred to untreated plants. Aphid samples were collected periodically for 5 to 7 d. All samples were analyzed for Rb content. Results were similar for the pepper and tomato experiments. Soil-drench treatment produced significantly greater levels of Rb in leaf and initial aphid samples relative to foliar treatment. Levels of Rb in aphids from the two treatments were comparable after aphids fed on untreated plants for 24 h. Levels of Rb in aphids declined rapidly after removal from the source: undetectable by Day 6 in the potato experiment and reduced by nearly 90% by Day 5 in the tomato experiment. Foliar application of RbCl solution should be used in field situations. Application is more precise, and the length of detection is comparable with soil drench. Additionally, soil drenching with Rb produced an initially high peak of Rb in the aphids, which may have a physiological effect.

KEY WORDS Rubidium, aphids, viruses.

Markers used to study insect populations should have minimal effects on the biology and behavior of the test insects (Southwood 1969). Since its introduction as a potential marker by Berry et al. (1972), rubidium (Rb) has been shown to be a desirable marker for several different orders of insects (Shepard and Waddill 1976, Burns et al. 1983, Fleischer et al. 1986, Payne and Wood 1984, Stimmann et al. 1973). Rubidium (as it occurs in RbCl) is low in natural abundance, non-radioactive, and nearly non-toxic to plants and animals, (Berry and Smith 1969, Berry et al. 1972). When applied in an aqueous solution, it is readily taken up by plants through the roots and leaves and translocated because it serves as a potassium surrogate (Wallace 1968, Levi 1970). Insects which feed on Rb-enriched plants are marked as well (Berry et al. 1972).

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Rubidium seems well suited to monitor the movement of aphids in the field, which would facilitate understanding of aphid ecology and epidemiology of plant viruses transmitted by aphids. Frazer and Raworth (1974) demonstrated that pea aphids (*Acyrthosiphon pisum* Harris) could be marked with Rb by maintaining the host plants (broad bean, *Vicia faba* L., and alfalfa, *Medicago sativa* L.) in nutrient solutions containing rubidium chloride (RbCl). Rubidium markers have been used to monitor the movement of other insects in the field (Stimmann 1974, Fleischer et al. 1986, Voss and Ferro 1990). Alverson et al. (1980) used Rb to simulate transmission of maize chlorotic dwarf virus by black-faced leafhopper, *Graminella nigrifrons* Forbes.

These experiments provide the basis for practical application techniques which will facilitate the use of a Rb marker to investigate aphid ecology and the epidemiology of aphid-transmitted plant viruses.

Materials and Methods

All experiments were conducted in the greenhouse at the University of Georgia in Athens January through March, 1991. Temperatures were maintained between 26°C and 29°C. In addition to natural sunlight, supplemental lighting was set on a 16:8 L:D h (high pressure sodium lamps, 400 watts, from P. L. Light Systems Canada, Inc.; Grimsby, Ontario). Two experiments were conducted with two aphid species and two species of host plants. The first experiment utilized bell pepper (*Capsicum annuum* Miller, 'Yolo Wonder') and green peach aphid (*Myzus persicae* Sulzer). Tomato (*Lysopersicon esculentum* L., 'Chico') and the potato aphid (*Macrosiphum euphorbiae* Thomas) were used in the second experiment.

In both the pepper and tomato experiments, the plants were grown from seed and transplanted into 20-cm pots in the greenhouse. All plants were fertilized weekly with a 20-20-20 water soluble fertilizer. When the plants were 8 weeks old, 15 robust plants of uniform size were selected. Five plants were sprayed to runoff with an aqueous solution of RbCl at a concentration of 10,000 ppm (10g/l), using a hand-held plant mister. None of the solution was allowed to contact the soil, but the soil was drenched with untreated tap water. The soil surrounding 5 other plants was drenched with a similar concentration of RbCl solution, and the foliage was misted to runoff with untreated tap water. The control group in each experiment consisted of 5 plants which received no Rb. The plants in the control group were misted to runoff with tap water, and the soil was drenched with tap water. All plants were watered 3 d after the experiment began.

Aphids for the experiments were taken from colonies maintained in the greenhouse on host plants of the same varieties used in the experiments. Forty-eight hours after the plants were treated with Rb, apterate aphids (500 to 1000 surviving aphids) were transferred to each plant using a small camel hair brush. After 6 days, the aphids were transferred from the treated plants to untreated plants of the same variety. On the same day, 5 aphid samples (80 to 100 aphids/sample) and 5 leaf samples (approx. 1 g of leaf tissue/sample) were collected from each experimental group (control, foliar application, soil drench). For the next 5 to 7 d, 3 to 5 samples (depending on available numbers of aphids)

were collected every 24 to 48 h from each group of aphids which had been transferred from the treated plants. The aphid and leaf samples were analyzed for Rb content using the methods described by Shepard and Waddill (1976). The relative Rb content of different experimental groups was compared using a student's *t* test.

Results and Discussion

Table 1 summarizes the results of Rb analyses of the aphids and host plants. In both the pepper and tomato experiments, a significantly greater mean concentration ($P < 0.05$) of Rb resulted in the leaves of plants in the soil drench treatments as compared with Rb levels observed following foliar application. A higher mean level of Rb in the leaves corresponded with an initially higher Rb concentration in the aphids. Aphids collected from plants which had received a soil drench had significantly greater mean levels ($P < 0.05$ in the tomato experiment; $P < 0.1$ in the pepper experiment) of Rb compared with aphids collected from plants receiving foliar application. Frazer and Raworth (1974) demonstrated a similar positive relationship between the concentration of Rb in *V. faba* marked with Rb and feeding aphids.

However, the difference in mean Rb content between aphids from the soil-treated plants versus foliar-treated plants was not significant ($P > 0.1$) in either experiment after the aphids had been removed from the source for 24 h or more. After 24 h, the mean level of Rb remained slightly higher in the aphids taken from the foliar-treated plants throughout the pepper experiment. Throughout the tomato experiment, the mean level of Rb remained slightly higher in the aphids collected from the plants receiving a soil drench.

Aphids which fed on plants receiving a soil drench of RbCl solution did not retain the Rb marker substantially longer than aphids taken from plants which were treated with a foliar solution (Table 1). By Day 6 after removal from the source plants, Rb was not detectable above background levels in the tomato/potato aphid experiment. The pepper experiment was not continued until Rb was undetectable because very few aphids remained on the plants beyond Day 5. However, by Day 5 after removal from the source plants, mean levels of Rb in the aphids had dropped more than 90% from initial levels. The mean level of Rb was slightly higher in the aphids taken from plants receiving foliar application of RbCl solution. Pea aphids feeding on V. *faba* in RbCl solution lost 77% of the Rb within 2 days of leaving the source plant (Frazer and Raworth 1974). Our data and those of Frazer and Raworth suggest that Rb is not a practical marker for aphids if the marker must be detectable for more than 4 to 5 days after the aphids leave the source.

For direct application of RbCl solution in the field, the results indicate that foliar application is preferable to soil drench. Foliar application is more easily made than a soil drench and can be directed more precisely. In the field, RbCl solution applied as a soil drench may move through the soil and be taken up by nontarget plants. Additionally, a soil drench of RbCl solution did not produce detectable Rb in the aphids for a substantially longer time than a foliar application.

Table 1. Levels of rubidium detected in aphids and foliage.

GREEN PEACH APHID/BELL PEPPER		POTATO APHID/TOMATO	
Application **Rb Content μg/g**[†]	Comparison degrees of freedom, *t* value	Application **Rb Content μg/g**	Comparison degrees of freedom, *t* value
LEAVES (collected directed from treated plants)			
Foliar (n = 5) **297.0 ± 132.2**	Foliar vs Control 8, 5.0*	Foliar (n = 4) **582.1 ± 130.9**	Foliar vs Control 7, 10.1*
Soil (n = 5) **974.1 ± 460.9**	Soil vs. Control 8, 4.7*	Soil (n = 3) **1510.8 ± 460.6**	Soil vs. Control 6, 7.8*
Control (n = 5) **1.68 ± 0.91**	Foliar vs Soil 8, 3.16*	Control (n = 5) **0.0 ± 0.0**	Foliar vs Soil 5, 3.16*
APHIDS (Day 0)[‡]			
Foliar (n = 5) **210.3 ± 61.5**	Foliar vs Control 8, 7.3*	Foliar (n = 5) **258.7 ± 92.0**	Foliar vs Control 8, 6.3*
Soil (n = 5) **336.1 ± 130.6**	Soil vs Control 8,5.6*	Soil (n = 3) **1691.6 ± 659.9**	Soil vs Control 6, 6.1*
Control (n = 5) **9.9 ± 4.3**	Foliar vs Soil 8, 1.95 (Significant, $P < 0.1$)	Control (n = 5) **0.4 ± 0.1**	Foliar vs Soil 6, 5.1*
Day 1			
Foliar (n = 5) **69.9 ± 13.5**	Foliar vs Control 8, 9.4*	Foliar (n = 5) **24.1 ± 1.2**	Foliar vs Control 6, 91.8*
Soil (n = 5) **58.8 ± 16.1**	Soil vs Control 8, 6.6*	Soil (n = 3) **52.7 ± 38.0**	Soil vs Control 6, 3.1*
	Foliar vs Soil 8, 1.2		Foliar vs Soil 6, 1.3
Day 5 (green peach aphid/pepper only on day 5)		**Day 4** (Potato aphid/tomato sample only on day 4)	
Foliar (n = 5) **22.4 ± 8.9**	Foliar vs Control 8, 2.8*	Foliar (n = 4) **3.4 ± 4.9**	Foliar vs Control 7, 1.4
Soil (n = 5) **20.4 ± 6.7**	Soil vs Control 8, 2.9*	Soil (n = 4) **7.6 ± 6.8**	Soil vs Control 7, 2.4*
	Foliar vs Soil 8, 0.4		Foliar vs Soil 6, 1.0
Very few aphids remained in the green peach aphid/pepper experiment beyond day 5; no additional samples were taken.		**Day 6, Day 7:** Rb not detectable above background levels in any treatment.	

* Significantly different, $P < 0.05$.
† g, wet weight.
‡. Days after removal from Rb treated plant. Day 0 aphids collected directly from treated plants.

A soil drench of RbCl solution did produce higher initial concentrations of Rb in the source plants and the aphids. However, this type of peak may be counterproductive. Greater resources are used, and some data suggest that higher levels of Rb may have undesirable physiological effects. Frazer and Raworth (1974) reported that Rb produced visible color change in pea aphid feeding on *V. faba* and may have been related to a reduction in fecundity.

Rubidium is detectable in aphids for a relatively limited time after they are removed from the source. This limitation, however, does not exclude Rb as a useful tool for exploring certain parameters of aphid ecology, particularly their role as vectors of non-persistent viruses. Many non-persistent viruses transmitted by aphids are retained for less than 1 day (see Sylvester 1954 for a review of aphid-transmitted non-persistent viruses). Alverson et al. (1980) demonstrated that Rb could be used to model the epidemiology of maize chlorotic dwarf virus, a non-persistent virus transmitted by black-faced leafhopper.

The ease and safety with which Rb may be applied to source plants create an extremely flexible experimental system with which to model aphid-transmitted viruses. Rb is readily absorbed through the foliage of many species of plants (Wallace 1968), and it is rapidly translocated throughout the plant (Levi 1970). The magnitude and number of the Rb source(s) could be adapted to simulate different conditions of initial viral infection (e.g., point source infection versus multiple infection sites); the epidemiology of the virus may be influenced by the initial infection. Furthermore, by marking plants near locations where marked aphids were captured, observers could simulate the spread of the virus through the field. The consistency among our experiments and the work of Frazer and Raworth (1974) suggest that the Rb would be a practical marker for wide variety of aphid/plant combinations.

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