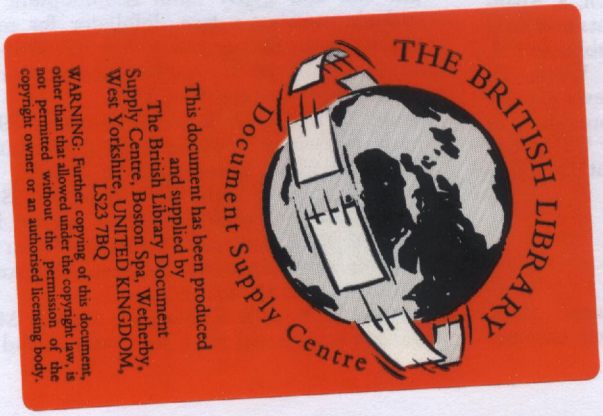

Kairomonal Baits: Effect on Acquisition of a Feeding Indicator by Diabroticite Vectors in Cucurbits

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ABSTRACT The influence of kairomonal baits, containing cucurbitacins, blossom volatiles, and carbaryl, on Diabroticite adult survivorship and trophic interactions with cucurbits was examined. Enrichment of cantaloupes using rubidium (Rb) was developed for monitoring trophic interactions. A soil-soak method was developed to enrich plant Rb concentrations. Beetle uptake of Rb followed a rectangular hyperbola and elimination after transfer to clean plants followed an exponential decay. Models showed beetle uptake occurred within 1 h and the mark is retained up to 2 wk. Naturally occurring endogenous Rb concentrations in beetles varied with species and sex. Baselines were established to allow determination of the incidence and intensity of beetle feeding on Rb-enriched plants. In field cages, kairomonal baits reduced the probability and intensity of feeding on cucurbits by *Diabrotica undecimpunctata howardii* (Barber) and *Acalymma vittata* (F.). In one trial, kairomonal baits totally blocked feeding interactions. In field plots, baits also significantly reduced feeding incidence and intensity in *D. u. howardii* and *D. virgifera virgifera* (LeConte), but not in *A. vittata*. Where the bait reduced feeding, males showed less reduction than females. In *D. v. virgifera*, there was a higher probability of capturing live males than females regardless of kairomonal treatment. Adult survivorship was reduced by the kairomonal bait in both field and cage experiments. These novel methods allow the monitoring of trophic interactions under field conditions in the presence of behavior-modifying semiochemicals. Hypotheses to explain variation among species and sex and implications of using kairomonal baits to manage vectoring of a pathogen are discussed.

KEY WORDS Diabroticite, cucurbitacins, rubidium

CUCURBITS ENCOMPASS CUCUMBERS (*Cucumis sativus*), melons (*Cucumis melo*), watermelon (*Citrullus vulgaris*), and squash and pumpkin (*Cucurbita pepo, C. moschata, C. maxima*). Together, these crops are an essential part of diversified farms (e.g., commercial production was present on 67% of Pennsylvania's vegetable farms in 1987). Two beetles, the striped cucumber beetle, *Acalymma vittata* (F.), and the spotted cucumber beetle, *Diabrotica undecimpuntata howardi* (Barber) are commonly found in cucurbits. Two related species, the northern corn rootworm, *D. barberi* (Smith & Lawrence), and the western corn rootworm, *D. virgifera virgifera* (LeConte), feed on the roots of grasses as larvae but invade cucurbits as adults. All of these beetles are in the taxon Diabroticina.

Cage studies (Rand & Enlows 1920) have demonstrated the ability of Diabroticites to vector *Erwinia tracheiphila* (E. F. Sm.) Holland, the causal agent of bacterial wilt, with losses of up to 75% (Sherf & MacNab 1986). The disease is more prevalent on *C. sativus* and *C. melo* than *Curcurbita* spp. Typically, inoculated leaves wilt within 5–6 d and the plant dies within 2 wk.

To acquire and transmit a pathogen, a vector must move into and feed on the crop. Trace element labeling is one technique of monitoring movement and trophic interactions (Akey et al. 1991). The concentration of an element (typically rubidium [Rb]) is enriched at one trophic level and is acquired at higher trophic levels as it moves through the food chain. Herbaceous plants have been enriched using foliar sprays (Shepard & Waddill 1976; Van Steenwyk 1978a, b; Legg & Chiang 1984; Fleischer et al. 1986, 1988). Acquisition of Rb from Rb-enriched baits (artificial nectar feeding stations) also has been demonstrated (Culin & Alverson 1986). Trace element concentration in the herbivore feeding on enriched host material is dependent on the time spent on that host and, thus, is correlated with feeding intensity. Acquisition of the enriched element by herbivores at concentrations significantly above endogenous levels indicates the occurrence of feeding on the host (Stimmann 1974). Rb may be acquired by feeding in a manner similar to pathogen acquisition by vectors and has been used as a pathogen surrogate to study

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transmission of maize chlorotic dwarf virus from johnsongrass to corn (Alverson et al. 1980a, b).

Feeding and movement behavior of Diabroticite beetles is influenced by cucurbitacins and blossom volatiles associated with cucurbit plants. Cucurbitacins are triterpenoid allomones primarily found in Cucurbitaceae and related taxa (Metcalf & Metcalf 1992). Cucurbitacin quality and quantity varies among plant species, tissue, and phenology (Metcalf & Lampman 1989) and production can also be induced by wounding (Tallamy 1985). The Diabroticites have turned these allomones into kairomones: cucurbitacins arrest beetle movement and are feeding stimulants (Metcalf et al. 1980; see Metcalf & Metcalf 1992 and Tallamy & Halaweish 1993 for a discussion of the adaptive significance of cucurbitacins to Diabroticites). Under laboratory conditions, effects on feeding behavior varied with age, reproductive status, sex, and prior exposure (Tallamy & Halaweish 1993). In cultivated species, cucurbitacins are not generally detectable in roots, undamaged leaves, and fruits, but cotyledons retain relatively high concentrations (Tallamy & Krischik 1989, Metcalf & Lampman 1989, Metcalf et al. 1982, Ferguson et al. 1983), and reduction to the stand at the early growth stage can be a major cause of crop loss (Brewer et al. 1987).

Cucurbitacins, however, are nonvolatile. The strong attraction of the Diabroticites to cucurbit flowers, however, suggests that other volatile compounds are used for longer distance attraction (Andersen & Metcalf 1986, 1987; see Metcalf & Lampman 1989 and Metcalf & Metcalf 1992 for a review). Beetle bioassays showed species specificity to particular blossom volatiles and volatile blends. A three-component mixture of 1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde showed a strong response of *Diabrotica* spp. and *A. vittata* (Lampman & Metcalf 1987, 1988; Lewis et al. 1990; Metcalf & Lampman 1989).

Knowledge about the chemical ecology of Diabroticites is leading to semiochemical formulations for pest management (Metcalf 1985). Baits impregnated with blossom volatiles, cucurbitacins, and small amounts of insecticide controlled rootworms in corn with little or no affect on predators (Weissling et al. 1991). Distribution of the granules within the corn canopy affected efficacy (Lance & Sutter 1991). In cucurbits, baits placed in petri dishes also demonstrated potential field efficacy. Addition of 1% eugenol resulted in a 4-fold increase in mortality of *D. u. howardii* (Metcalf et al. 1987). Semiochemical bait technology in field corn, however, will not be directly transferable to cucurbits. Among the Diabroticites, sensitivity to the nonpheromonal attractants and cucurbitacins varies by orders of magnitude (Metcalf & Lampman 1989). Also, the stage of the pest for which control is required differs: vine crops require control of adults, often early in the cropping season, as opposed to late-season applications in field corn to control F_1 larvae. Crop canopy architecture, which affects bait distribution, is also very different. Furthermore, periodic applications to control prolonged adult emergence or immigration is less economically competitive in field corn than in cucurbits.

The present approach to managing Diabroticites in cucurbits has little tolerance for the beetles because of their ability to transmit *E. tracheiphila*. Growers have relied on a preplant application of systemic carbofuran, but this material has demonstrated leaching capabilities, reduced effectiveness in soils with a history of use (Buhler et al. 1992), avian mortality in the granular formulation, and is highly toxic (acute rat oral LD_{50} of 11 mg/kg). Alternatively, growers use expensive fabric covers (Adams et al. 1990) or repeated sprays of insecticides at the first sight of beetles. Fresh-market cucurbits are hand harvested, and protection from rind-feeding late in the season with insecticides conflicts with ensuring farm worker safety. Cucurbitacins are toxic plant-derived compounds (Metcalf 1985), but formulated products of kairomonal baits (including the carbaryl) have an acute rat oral LD_{50} of >5,000, an acute dermal LD_{50} of >2,000, no inhalation hazard, and the safest categorization possible for primary eye and dermal irritation (J. Gaggero, personal communication). Semiochemicals affect beetle movement and feeding behavior and, thus, could affect vectoring potential and could provide significant improvements for farm worker safety. The objective of this article was to investigate the potential of using Rb-enrichment technology to monitor the influence of semiochemical baits on trophic interactions and short-term adult survivorship of Diabroticites in field-grown cucurbits.

Materials and Methods

Rubidium Introduction. Three techniques of introducing Rb into 3-wk-old cantaloupe seedlings ('NK Rodgers NVH896') were compared in a greenhouse: (1) stem injection of 150 ml of a 3,000 ppm Rb solution injected with a syringe at five locations of the stem, (2) foliar spray until runoff with a 15,000 ppm Rb solution, and (3) soaking the potting media (a mix of peat, perlite, and vermiculite; Metro-Mix 250, Grace Sierra, Milpitas, CA) with 100 ml of a 15,000 ppm Rb solution, placing a tray under the pots to collect leachate, and allowing the plants to absorb the leachate. All solutions were made from the RbCl salt. Each technique was applied to five plants, and five untreated plants served as controls. Treatments were applied on 23 April 1992, and the foliar spray and soaking treatments were applied to the same plants a second time on 28 April. Plants were allowed to grow in the green-

house until 8 May, at which time leaf, flower, and stem tissue was frozen for later determination of Rb concentrations. Concentrations were determined from 77 samples, and differences in Rb concentrations among techniques and plant parts was analyzed with a two-way ANOVA. *T*-tests were used to compare means.

Based on these tests, a modification of the soil soaking technique was used to prepare transplants for field studies. Cantaloupe seeds (NK Rogers NVH896) were planted in the greenhouse on 27 May 1992 and grown to the two-leaf stage (≈3-wk-old plants) in six-pack plastic containers, and seven containers placed into plastic trays. A 4,000 ppm Rb solution made from the RbCl salt was poured into the trays at a rate of 25 ml per transplant on 16 and 18 June, and the seedlings and potting mix were allowed to imbibe the solution for up to 5 d. Plants were then watered once with tap water before transplanting on 23 June (see field experiments, below). The Rb concentrations of foliar tissue in these plants was determined on 18 June (before transplanting), and from plants grown under field conditions on 26 June, 10, 16, 24 and 31 July, and 20 August. Concentration was plotted against days after initial introduction of Rb. Foliar concentration was compared between treated and control plants on the last sampling date using a one-way ANOVA (n = 43). Additionally, the Rb concentration of flower tissue was determined on 20 August, and differences among plant parts examined with a one-way ANOVA (n = 30 for flower tissue and 43 for leaf tissue).

Rubidium Baselines. Endogenous Rb concentrations were determined in 436 Diabroticite beetles representing all four species commonly found in cucurbits collected in the field from four locations, at distances of 1 to >10 km from the study site in 1992 and 1993. In addition, the Rb concentrations in 25 male and 18 female *D. u. howardii* from a colony reared at Dupont (Steine Laboratory, Newark, DE) was determined. Beetles from Dupont had been reared on corn root seedlings as larvae, and provided with artificial diet as adults. Rb concentrations were determined for each species and sex for both field-collected and colony-reared beetles. Differences in mean concentrations between sexes for each species were compared with a one-way ANOVA (n ranged from 18 to 88 per species and sex), and conformity with a normal distribution tested using the Shapiro–Wilks' statistic (PROC UNIVARIATE, SAS Institute 1989). A baseline for each species, sex, and rearing source, above which a beetle was categorized as containing Rb concentrations in excess of endogenous levels, was calculated as the mean plus three standard deviations (Stimmann 1974).

Uptake and Elimination of Rubidium. The rate of uptake of Rb in *D. u. howardii* was determined from beetles taken from the Dupont colony. Beetles (37 females and 39 males, unknown ages) were caged on transplants that had been enriched with Rb as described for the transplants used in the field experiments. Live adults were collected at 0, 1, 4, 5, 6, 7, and 11 d after caging on the Rb-enriched plants. The mean Rb concentrations of these beetles was expressed as a rectangular hyperbolic function of days caged on Rb-enriched host (n = 7 to 14 per day) using the secant method of nonlinear regression (PROC NLIN, SAS Institute 1989).

The rate of elimination was determined from *D. u. howardii* taken from our laboratory colony which was reared in the same manner as that used in Dupont. Cantaloupe seedlings (NK Rogers NVH896) were enriched with Rb as described in the soil-soak method, using 25 ml per transplant of a 4,000 ppm Rb solution from the RbCl salt applied twice, allowing the seedlings to imbibe the solution for 5 d, and rinsing with tap water before caging beetles. Six packs of 5-wk-old enriched transplants were then placed in 0.03 m^3 cages. Three cages were established using seven packs per cage, and 70 adults <3 d old were placed in each cage. Cages were held at 26°C and a photoperiod of 16:8 (L:D) h. Beetles were allowed to feed for 7 d to ensure good uptake of the marker, and then transferred to clean seedlings. Beetles were collected and frozen at 0, 0.5, 1, 2, 3, 7, 16, and 24 d after transfer to clean seedlings. Also, at the time of caging, an additional 20 beetles were collected and frozen from the colony. The mean Rb concentrations of beetles removed from enriched host material was expressed as an exponential decay function of the number of days taken from the Rb-enriched host (n = 11 to 16 per day with the exception of the 24-d determination, where n = 1) using the secant method of nonlinear regression (PROC NLIN, SAS Institute 1989).

Cage and Field Experiments. Experiments were conducted to assess the impact of kairomonal baits on Diabroticite adult survivorship and feeding, using Rb as a marker to measure incidence and intensity of feeding on plants under field conditions. A replicated complete block experiment, using six replications of two treatments, was established on 23 June 1992 at Rock Springs, PA. Plots were 58 m^2 placed on the edges of sweet corn fields. Each plot had four rows of cantaloupes with plastic mulch and drip irrigation on 1.5-m centers, and plants were placed 0.6 m within each row. Rb-enriched transplants were used for all plants in both treatments. A small amount of soil was placed on top of the potting media at transplanting to minimize the potential of acquisition of Rb by beetles from the soil as opposed to feeding on the plants. Treatments consisted of an untreated control, and an 11.4-kg formulated product per hectare (10 lb/acre) rate of a granular kairomonal bait (SLAM, MicroFlo, Lakeland, FL). The

kairomonal bait consisted of a corn-cob grit base with carbaryl (1.3% wt:wt), buffalo gourd root powder containing cucurbitacins E and I, and volatile attractants (1,2,4 trimethoxy benzene, indole, cinnamaldehyde, 4-methoxy cinnamaldehyde, cinnamyl alcohol, 3-phenol propanol, and 4-methoxy phenethanol) adhered to this base. Releases of *D. u. howardii* from the Dupont colony were made to augment natural populations on 25 and 30 June and 8 July. Releases consisted of two cartons of 40 adults (unknown age and sex) per carton per plot. These beetles were obtained from DuPont the day before release and held in 1-liter waxed paper cartons with two damp paper towels overnight without food. They were released the morning following by placing each carton on its side between two rows of plants and pulling the lid to one side. Most of the beetles left the cage within a few hours. Those that remained in the carton at the end of that afternoon (less than five) were gently tapped from the carton onto plants in the plot.

Kairomonal bait was applied approximately weekly (24 and 30 June; 8, 15, 22, and 29 July; and 5 August) with a hand-pushed granular applicator. On dates when baits were applied and augmentative releases of beetles were made, the bait was applied before releasing beetles. Samples were collected from five consecutive plants, two samples per plot, 1 or 2 d, or both, after each application except after the 30 June application because of rain. Live and dead Diabroticite adults (*A. vittata, D. u. howardii, D. barberi,* and *D. v. virgifera*) were counted, collected, and frozen from a 0.4-m^2 area centered around each plant in these sample areas on 26 June, 9, 16, 17, 23, 24, 30, and 31 July, and 6 August. On some dates it was difficult to find live beetles in some plots. When the total number of live beetles collected from a treatment was <10, all plots for that treatment were searched for live beetles for about 5 min. Plant tissue was collected and frozen from these plants on 26 June, 10, 16, 24, and 31 July, and 20 August.

The influence of the kairomonal baits on beetle mortality and feeding was also measured in cages placed in these plots. One hardware cloth cage (1–2 m^3) was placed on top of one plant in each of the five treated and five control plots described above on 9, 14, 22, and 28 July. Before placement, plants were inspected to ensure that no adult Diabroticites were present, and the base of the plants screened to prevent escape beneath the plastic mulch. Kairomonal baits were applied before cage placement as described above. On the first three dates, 10 adult *D. u. howardii* taken from the Dupont colony were aspirated into each cage. On the last date, 10 *A. vittata* collected from nearby farms were aspirated into the cages. One and 2 d following the application of bait, the number of live and dead beetles were counted. Dead beetles were removed as they were found, and all beetles were removed after 2 d. The beetles collected from the cages were frozen, and their Rb concentrations determined.

For both field and cage studies, the Rb concentration of each beetle was compared with the appropriate baseline for that species and sex determined from the endogenous beetles, and categorized as marked or non-marked. Also, each beetle was categorized as living or dead at the time of collection. Because these data were categorical (e.g., data were either above or below baseline, or live or dead), the frequencies were analyzed with categorical modeling (Freeman 1987). For each species (n ranged from 45 to 80 in the caged studies, and from 86 to 271 in the field studies except for *D. barberi,* which had small samples sizes of 11 to 26), the significance of treatment (kairomonal bait), sex, and the interaction of treatment and sex on the probability a beetle being categorized as marked or alive 2 d after treatment was determined with the G-statistic calculated with the general linear solution of categorical modeling (PROC CATMOD, SAS Institute 1989). Additionally, mean Rb concentrations of beetles collected from cages with and without kairomonal bait was compared with a one-way ANOVA, and mean Rb concentrations of beetles collected in the field plotted over time.

To determine if the Rb might have leached from enriched plants into the kairomonal bait, Rb concentrations in seven samples of bait collected before field application was compared with 19 samples taken from field plants on 25 June using a one-way ANOVA.

Rubidium Determinations. Beetles were sexed, dried at 60°C for 3 d and dry weights determined. Plant tissue and kairomonal baits were also dried at 60°C for 3 d and 0.025 g dry weight used to determine Rb concentrations. All data were expressed on a parts-per-million dry-weight basis. A microwave digestor (MDS-81D, CEM, Mathews, NC) was used to wet ash samples. Samples were ashed in 15-ml conical polypropylene screw-cap tubes with the caps screwed on. For each batch processed, eight tubes for the beetle samples, and six tubes for the plant samples, were held upright using a 600-ml glass beaker. Individual adult beetles were ashed in 0.3 ml of HNO_3 for 86 s at 26% power of the magnetron. For the dry plant tissue, samples were ashed at 115 s at 10% power. The magnetron provided 670 ± 13 W (n = 3, measurements made at monthly intervals). After ashing, solutions were brought up to 1 ml with distilled water, and Rb concentrations determined using the emission mode of a Video 22 atomic absorption spectrophotometer (Thermo Jarrell Ash, Franklin, MA) set at 780 nm. Four estimates of each milliliter of solution were made by the spectrophotometer without delay, and the mean used to estimate concentration.

Table 1. Rb concentrations (ppm dry weight) in different cantaloupe plant parts after three Rb introduction methods

Plants	Days after Rb-introduction	Rb-introduction method	Plant part		
			Flower	Leaf	Stem
Greenhouse	14	Control	59b[a]	73ab	110a
		Inject stems	976ab	393b	1,056a
		Spray foliage	14,000b	41,814a	12,876b
		Soak soil	88,700b	146,122a	115,081ab
Field-grown	65	Control	51a	40b	—[b]
		Soak soil and transplant	209a	129b	—

[a] Means among plant parts followed by the same letter are not significantly different, least significant difference (LSD) test, $P > 0.05$.
[b] —, No data.

Results

Plant Rubidium Concentrations. All Rb-introduction methods elevated concentrations of the trace element in transplants 14 d after initial introduction (Table 1). Among the three methods tested, soaking the potting media resulted in highest concentrations ($n = 77$, $P < 0.001$) regardless of the plant tissue. Thus, this method was developed to prepare transplants for the field. However, yellowing of the foliage was observed in plants soaked twice with 100 ml of a 15,000 ppm Rb solution, and host material of this high of a Rb concentration has been shown to reduce fitness of arthropods. Because of this phytotoxicity and the need to provide a Rb-enriched host that would not be expected to reduce fitness, transplants for field studies were soaked twice with a reduced volume and concentration (25 ml of a 4,000 ppm Rb solution), and they were watered once with tap water before transplanting. This resulted in foliar concentrations in the transplants of 16,321 ± 2088 SEM ppm Rb ($n = 21$) on 26 June (3 d after transplanting). Foliar concentrations of transplants used in the field followed an exponential decay with time (Fig. 1), approaching endogenous concentrations 40–45 d following initial soaking.

Rubidium concentrations varied among plant parts (Table 1). Before enrichment, stem tissue contained higher concentrations than flower tissue. Enrichment changed the relative allocation of the trace element within the plant: spraying foliage or soaking the potting media resulted in higher concentrations in leaves than flowers, and injecting stems elevated concentrations in stems relative to foliage. Plants closer to maturity (65 d after transplanting), however, had higher Rb concentrations in the flowers in both enriched and control plants. Presumably, the Rb was translocated to the reproductive tissue as the plant matured.

Rubidium did not leach into the kairomonal granules. Mean concentration in granules taken before field application (17.3 ± 0.5 ppm) did not differ from those collected from plants in the field (18.1 ± 0.9 ppm) ($n = 26$, $P > 0.62$).

Rubidium Baselines. In naturally occurring endogenous populations, mean concentrations of Rb varied among beetle species. Concentrations were significantly higher in females than males in three of four Diabroticite species (Table 2). The pattern of higher female concentrations is consistent with other reports (Fleischer et al. 1986, 1989; Knight et al. 1989; Stimmann 1973; Graham & Wolfenbarger 1977; Van Steenwyk et al. 1978; Legg & Chiang 1984). Baselines, used to distinguish insects that have fed on Rb-enriched plants from endogenous populations, have generally been given as the mean plus

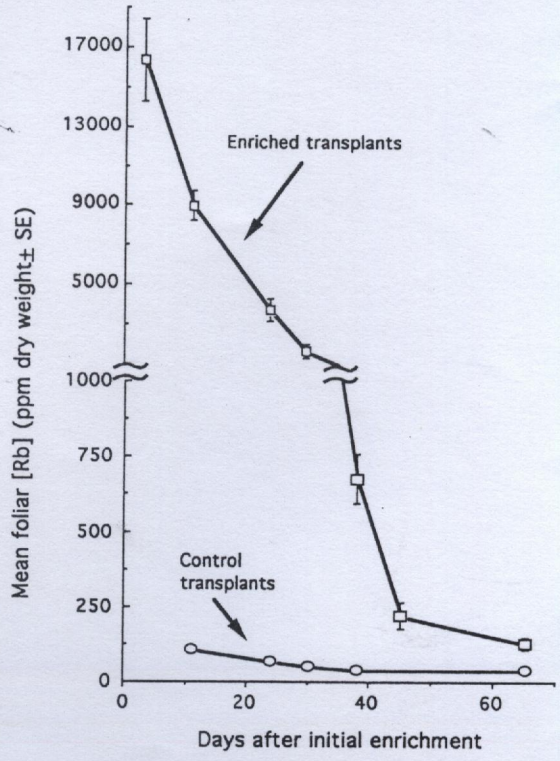

Fig. 1. Foliar Rb concentrations (ppm dry weight) in *C. melo* enriched with two applications of a 4,000-ppm Rb soil soak before transplanting.

Table 2. Endogenous Rb concentrations (ppm dry weight) in four species of Diabroticite beetles

Species	Source	Sex	*n*	Mean	SD	Baseline
D. u. howardii	Colony	F	18	57.01a[a]	47.00	198.01
		M	25	33.07b	32.85	131.62
		Combined	43	43.09	40.67	165.09
	Field	F	26	29.70a	18.24	84.42
		M	73	17.53b	7.59	40.30
		Combined	99	20.77	12.55	58.42
D. barberi	Field	F	36	18.62a	4.59	32.39
		M	30	15.44b	4.56	29.12
		Combined	66	17.18	4.81	31.61
A. vittata	Field	F	46	18.52a	5.64	35.44
		M	82	16.24a	10.95	49.09
		Combined	128	17.06	9.43	45.35
D. v. virgifera	Field	F	88	14.19a	7.10	35.49
		M	55	10.31b	3.86	21.89
		Combined	143	12.70	6.33	31.69

[a] Means for the two sexes of a given species and collection source followed by the same letter are not significantly different, ANOVA, $P > 0.05$.

three standard deviations determined from the endogenous population (Stimmann 1974). Given a normal distribution, this should result in a baseline that exceeds 99.7% of the Rb concentrations in endogenous beetle. Because of the differences among species and sex, a unique baseline was established for each. Using field-collected beetles, baseline concentrations ranged from 22 to 85 ppm. With the exception of male and total *D. barberi*, Rb concentrations in adult Diabroticites deviated from a normal distribution ($P < 0.01$, Shapiro–Wilks' statistic to test for normality). Even in the presence of the skewed frequency distributions, however, these baselines exceeded endogenous concentrations found in 95 and 97% of the field-collected male and female *D. u. howardii*, 97% of male *A. vittata*, 99% of female *D. v. virgifera*, and 100% of all others. *D. u. howardii* from the colony had greater standard deviations, which resulted in higher baselines. Different baselines may reflect different feeding habits, because individuals collected from different hosts acquire different Rb concentrations, even when collected from the same geographic area (Fleischer et al. 1986).

Uptake and Elimination of Rubidium. Rb concentrations in *D. u. howardii* adults increased asymptotically with time when caged on the same Rb-enriched cantaloupes used in the field studies (Fig. 2). The uptake curve was modeled as a rectangular hyperbola, $y = A * x/(B + x)$, where y is the parts per million of Rb per beetle on a dry-weight basis, x is days caged on Rb-enriched host, and A and B are regression coefficients. Confidence intervals for the regression coefficients for each sex overlapped; therefore, the regression was fit to the data from both sexes. Using nonlinear regression, the regression coefficients (±SE) were 14,911 (±2,061) for A and 3.3 (±1.27) for B ($R^2 = 0.92$). Assuming a baseline of 166 ppm from *D. u. howardii* beetles in the colony, this implies that *D. u. howardii* acquires sufficient Rb to be considered marked after 54 min of being caged with enriched cucurbits. Assuming equal uptake rates, and a baseline of 50 ppm, which was more typical of field-collected populations, only 16 min on enriched host would result in a mark. Foliar Rb concentrations in the host plants used in the cages during the study ranged from 16,321 (±2,088, $n = 21$) on 26 June (i.e., 1 d after caging), to 13,556 (±1,462, $n = 19$) on 10 July (i.e., 14 d after caging). Lower plant concentrations may require greater exposure time to achieve a mark.

Rubidium concentrations in beetles caged on similar Rb-enriched plants for 7 d and then transferred to clean plants declined in an exponential decay pattern. This was modeled as $y = C * \exp(D * x)$, where C and D are regression coefficients, y is the mean rubidium concentration, and x is days after removal from enriched host

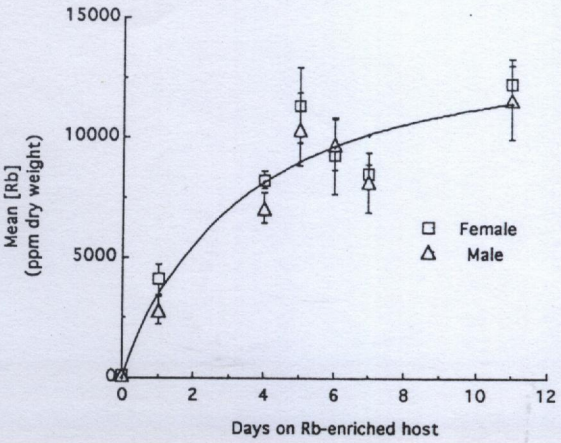

Fig. 2. Uptake of Rb in *D. u. howardii* caged on Rb-enriched cantaloupe transplants. Uptake followed the hyperbolic model $y = 14{,}911 * x/(3.3 + x)$, with an R^2 of 0.92.

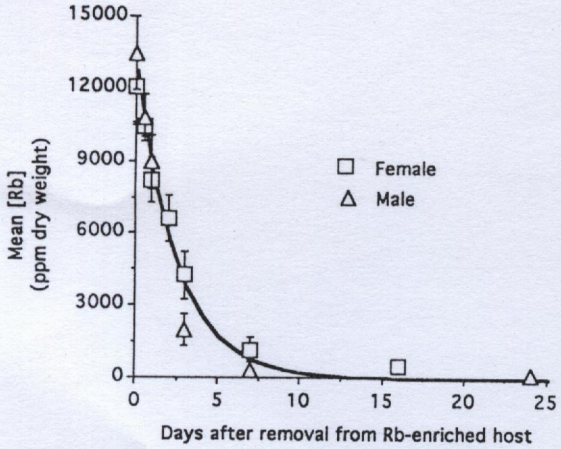

Fig. 3. Elimination of Rb in *D. u. howardii* after being caged on Rb-enriched transplants for 7 d and then transferred to clean transplants. Elimination followed an exponential decay model $y = 12{,}789\ e^{(-0.4 * x)}$.

(Fig. 3). Coefficients overlapped between sexes, so a single curve was fit to the data, resulting in estimates (±SE) of 12,789 (±458) for C and −0.4 (±0.04) for D ($R^2 = 0.99$). Assuming a baseline of 50 ppm, this implies that mean concentrations from enriched adults could be distinguished from endogenous populations for almost 2 wk. Variation about the means would probably preclude reliance on the ability to distinguish marked adults for more than ≈10 d.

Caging Experiments. The proportion of beetles exceeding baselines was very high in cages placed in control plots: proportions ranged from 79 to 100%, and exceeded 96% in three of four trials (Table 3). Placing cages in plots treated with kairomonal bait significantly reduced this proportion in all trials, which included three with *D. u. howardii* and one with *A. vittata* ($P < 0.05$). However, the degree of reduction varied. The most dramatic reduction occurred in the third trial with *D. u. howardii*, where 100% of the control beetles were marked compared with none of the beetles in cages placed in plots treated with kairomonal baits. In this trial the kairomonal bait totally blocked any feeding of the beetles with the crop. In other trials, however, up to 64% of the beetles in cages placed within plots treated with kairomonal baits acquired sufficient Rb to be considered marked. The influence of kairomonal bait on the proportion of beetles carrying a mark was not affected by sex in any of these cage trials ($P > 0.16$), and there was no sex by treatment interaction ($P > 0.45$).

Survivorship was also high in the control plots: in three of four trials 100% of the beetles in the control plots were recaptured alive (Table 3). The third trial with *D. u. howardii*, however, resulted in only 59% survivorship in the controls. This survivorship was significantly reduced by the kairomonal bait in all trials ($P < 0.01$) but, as in the measure of feeding incidence, the degree of reduction varied. In three of the four trials, involving both *D. u. howardii* and *A. vittata*, only 0–12% of the beetles in the treated plots were alive after 48 h. In the first trial, however, 52% of the *D. u. howardii* beetles in the treated plots were alive after 48 h. Sex was not a significant factor influencing the proportion of beetles alive after 48 h ($P > 0.07$ in trial 3, and $P > 0.52$ in the remaining three trials), and there was no sex by treatment interaction ($P > 0.56$).

The Rb concentration in beetles taken from cages in control and treated plots declined over time (i.e., from the first to the fourth trial) (Table 4), reflecting the reduction in plant concentrations (Fig. 1). The kairomonal bait did not effect Rb concentration in live *D. u. howardii*, but it resulted in >1 order of magnitude reduction of Rb concentration in live *A. vittata* (Table 4). However, beetles were not generally recaptured alive from treated plots, and the mean Rb concentration in live beetles from treated plots is based on a small sample size in most trials. Among beetles that were dead after 48 h, mean concentrations remained comparable to live beetles when they were collected from control plots but were reduced dramatically when taken from plots treated with kairomonal bait. Although there were exceptions, the caged trials were characterized by control plots containing mostly live beetles with high Rb concentrations, and treated plots containing mostly dead beetles with low Rb concentrations.

Table 3. Kairomonal bait treatment effect within cages upon incidence of feeding (the percentage of beetles carrying an Rb mark above endogenous levels) and survivorship during 2 d

Species	Trial	No. collected	% Marked (±SE)[a]		% Alive (±SE)[a]	
			Control	Treated	Control	Treated
D. u. howardii	1	50	96 (.04)	64 (.10)*	100 (.03)	52 (.10)**
	2	45	79 (.09)	19 (.08)***	100 (.04)	12 (.06)***
	3	80	100 (.00)	0 (.00)***	59 (.07)	0 (.02)***
A. vittata	4	78	97 (.03)	41 (.07)***	100 (.02)	5 (.03)***

[a] Estimate of SE and comparison between control and treated cages using G-statistic from categorical modeling. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4. Mean Rb concentration (ppm dry weight) in beetles recaptured from cages placed in plots treated with kairomonal baits and control plots. (number in parentheses is the sample size)

Species	Trial	Recaptured alive		Recaptured dead	
		Control	Treated	Control	Treated
D. u. howardii	1	1,752 (25)a	1,443 (13)a[a]	— (0)	79 (12)
	2	439 (19)a	311 (3)a	— (0)	22 (23)
	3	546 (26)	— (0)	373 (18)a	15 (36)b
A. vittata	4	311 (34)a	22 (2)b	— (0)	94 (44)

[a] Means between control and treated plots for a given mortality class followed by the same letter are not significantly different, $P > 0.05$, ANOVA.

Field Trials. As in the caged trials, beetles from the control plots had a high probability of containing an Rb concentration that clearly demonstrated they fed on Rb-enriched plants. Modeled proportions of marked beetles ranged from 74 to 98%, and these estimates were within one standard error of 95–100%, respectively (Table 5). Addition of the kairomonal bait significantly reduced the probability of plant-feeding (i.e., reduced the probability of carrying an Rb mark) in *D. u. howardii* and *D. v. virgifera*. ($P < 0.02$) (Table 5). In these two species, where the bait had a significant impact on feeding incidence, sex was also a significant factor ($P < 0.001$), with males showing less reduction in the incidence of plant feeding than females. In *D. barberi* and *A. vittata*, however, there was no significant impact of kairomonal treatment on the proportion of marked beetles ($P > 0.05$) (Table 5). There was a numerical reduction in *D. barberi* (from 90 to 63% in females, and 80 to 42% in males). *D. barberi* population densities were low, and it is possible that the statistical test lacked the power to distinguish a significant difference. In *A. vittata*, however, sample sizes were high, and beetles had virtually identical probabilities of carrying an Rb concentration in excess of endogenous beetles in both control- and bait-treated plots (96 and 95% in females, and 93 and 93% in males, respectively). Clearly, virtually all *A. vittata* individuals exhibited feeding behavior in the field, regardless of whether they were in a treated or control plot (and regardless of whether they were collected alive or dead, see below). Thus, the influence of the kairomonal bait on feeding behavior differed in *A. vittata* compared with *Diabrotica* spp. In the species where the bait had no significant impact on feeding incidence (*D. barberi* and *A. vittata*), sex also had no significant influence ($P > 0.05$). There was no sex by treatment interaction in the incidence of feeding in any of the four species ($P > 0.45$).

Although the bait had a variable effect on the incidence of feeding, it had a significant ($P < 0.01$) and consistent effect in adult survivorship. In all four species, adult survivorship was high in the control plots, with modeled estimates ranging from 73 to 98%, and within one standard error of 95 to 100%, respectively. Addition of the bait reduced the probability of capturing adults alive to between 12 and 30%. Sampling was biased toward recapturing live beetles (additional time was spent looking for live beetles when they were not found within the two five-plant sample units in order to have enough beetles to estimate feeding incidence). Thus, estimates of the proportion of live beetles in treated plots are probably overestimates.

In three species, sex was not a significant factor in the proportion of live beetles in control or

Table 5. Influence of kairomonal bait and sex on survivorship and the probability of the Rb concentration in a beetle exceeding the baseline defining endogenous populations

Species	Sex	No. collected	Probability ($\times 10^{-2}$) of being marked (±SE)[a]		P[b]		Probability ($\times 10^{-2}$) of survivorship over 2 d (±SE)[a]		P[b]	
			Control	Treated	Treatment	Sex	Control	Treated	Treatment	Sex
D. u. howardii	F	107	93 (04)	20 (05)	0.0001	0.0001	98 (02)	20 (05)	0.001	NS
	M	163	98 (01)	51 (04)			98 (02)	22 (04)		
D. barberi	F	11	90 (10)	63 (16)	NS	NS	84 (16)	20 (12)	0.01	NS
	M	26	80 (19)	42 (10)			86 (14)	23 (09)		
A. vittata	F	198	96 (02)	95 (02)	NS	NS	100 (01)	31 (04)	0.001	NS
	M	271	93 (02)	93 (02)			99 (01)	22 (03)		
D. v. virgifera	F	86	74 (21)	19 (04)	0.02	0.001	73 (22)	12 (04)	0.007	0.002
	M	172	89 (11)	40 (04)			89 (10)	30 (04)		

[a] Probability estimated using categorical modeling (Proc Catmod; SAS Institute 1989). Residual chi-square $P > 0.45$.

[b] For each species, the probability of a lower G-statistic comparing probabilities among treatments (control versus kairomonal bait) and sex (females versus males). Residual chi-square showed that treatment * sex interactions were not significant ($P > 0.45$ to 0.78). NS, Not significant for treatment or sex, $P > 0.05$.

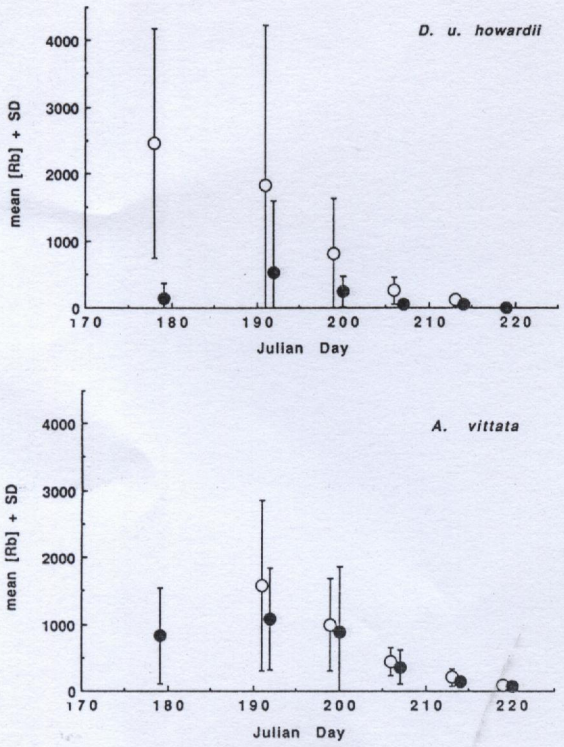

Fig. 4. Rb concentrations (ppm dry weight) over time from *D. u. howardii* and *A. vittata* collected from Rb-enriched plants under field conditions. Open circles, control plots; closed circles, plots treated with kairomonal baits.

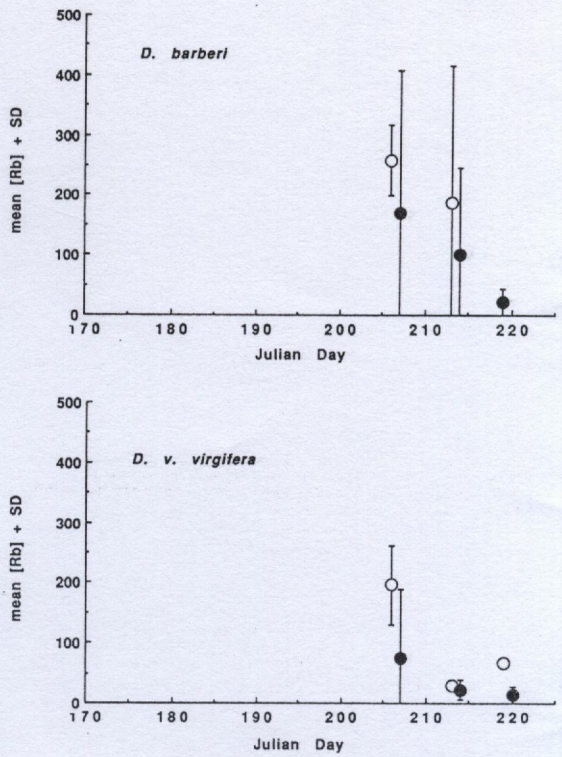

Fig. 5. Rb concentrations (ppm dry weight) over time from *D. barberi* and *D. v. vergifera* collected from Rb-enriched plants under field conditions. Open circles, control plots; closed circles, plots treated with kairomonal baits.

kairomone-treated plots. In *D. v. virgifera*, however, there was a higher probability that males, rather than females, would be captured alive. There was no sex by treatment interaction ($P > 0.62$). The increased tendency to capture male *D. v. virgifera* was not caused by the kairomonal bait, but was found in both treated and control plots.

Rubidium concentrations also reflected the decreasing plant concentrations over time (Fig. 4 and 5). Concentrations are separated for beetles from treated versus control plots, but pooled for live and dead beetles within these plots. The impact of kairomonal baits on these concentrations suggested variation among species similar to the data reporting the probability of feeding on the plant. Two species, *D. u. howardii* and *A. vittata*, were present throughout the field season (Fig. 4). In *D. u. howardii*, where the incidence of feeding was reduced by the kairomonal bait, concentrations were generally lower in beetles from treated plots. But in *A. vittata*, where the bait reduced survivorship but not feeding incidence, the concentrations in treated plots (made up primarily from dead beetles) mirrored those from control plots (made up primarily of live beetles). Thus, for *D. u. howardii*, the bait affected both survivorship and tropic interactions of the beetles with the plant but, for *A. vittata*, the beetles fed on the plants before being killed. The two species that are typically associated with corn, *D. barberi* and *D. v. virgifera*, invaded the plots later in the season. Plant concentrations had dropped to much lower levels (Fig. 5, note the range in the *y*-axis is almost an order of magnitude less than Fig. 4), making it more difficult to separate the effect of the bait on feeding intensity.

Discussion

Rubidium Dynamics. Data presented here show that it is feasible to enrich cucurbits with Rb and then transfer this mark to Diabroticite beetles. The soil-soak method helped ensure that transfer to the herbivore occurred through feeding. The kairomonal bait was not contaminated with Rb. Thus, if the beetle had enriched Rb concentrations, it came from feeding on the plant, not from leaching into the bait and then feeding on contaminated bait. Phytotoxicity was not observed in these studies, but delayed growth and yellowing of another cultivar has been observed in subsequent experiments (un-

published data). Rb-induced phytotoxicity in cucurbits may vary with cultivar and weather conditions, which differed in these subsequent studies. Lower rates and repeated soil-injections can also achieve season-long Rb enrichment in cucurbits, but delayed early growth may remain a symptom of plant stress (unpublished data). Reduced fitness of herbivores has been negligible when concentrations in the first trophic level remained below ≈15,000 ppm (Stimmann et al. 1973, Van Steenwyk et al. 1978a, Knight et al. 1989), which covers most of the range exhibited by the plants in the experiments reported here (Fig. 1).

Herbivore uptake and elimination of trace elements were dynamic with time, and define constraints within which it is feasible to use the technology for a given insect/plant system. Uptake in adult *D. u. howardii* caged on enriched cucurbits was rapid and asymptotic with time. Adults obtained sufficient Rb to be considered marked within an hour of access to enriched host. Field data from within cages and in open plots showed that a very high proportion of four species of Diabroticite beetles collected from Rb-enriched plants contained enriched levels of Rb. Thus, the potential of marking the vast majority of beetles in a field plot with Rb was clearly demonstrated. Elimination of Rb in *D. u. howardii* followed an exponential decay similar to the pattern observed when only the adult stage was allowed access to enriched host material in other Coleoptera (Shepard & Waddill 1976, Wolfenbarger et al. 1982, Voss & Ferro 1985) and Heteroptera (Fleischer et al. 1986). Presumably, the Rb was primarily in the gut, and assimilation into insect tissue during the adult stage was limited. Allowing access to Rb-enriched host material during the larval stages may result in greater assimilation, and a mark that lasts for a longer time. In the laboratory studies, sufficient concentrations to be considered marked remained in *D. u. howardii* for 10–14 d after transfer from enriched host material. Studies of movement would need to take this constraint into consideration (e.g., Fleischer 1988) or develop methods that result in marks that last for a longer time. However, for purposes of determining the influence of kairomonal pesticides on trophic interactions and potential acquisition of a pathogen, the elimination rate is not a constraint, because the Diabroticites were collected directly from the Rb-enriched host.

Impact of Kairomones. Caged studies showed that kairomonal baits have the potential to reduce feeding incidence significantly (the probability of acquiring sufficient Rb to be considered marked), feeding intensity (Rb concentrations) and adult survivorship of both *D. u. howardii* and *A. vittata*. In one of four caged trials, the bait totally blocked feeding interactions between the herbivore and plant. Under field conditions, the bait was also successful at dramatically reducing adult survivorship of these two species and *D. barberi* and *D. v. virgifera*. But the field studies revealed a dramatic variation among species in the impact of the bait on feeding incidence and intensity. Relative to three *Diabrotica* species, *A. vittata* was more likely to feed upon cucurbit plants in the presence of these kairomonal baits.

This variation among species may be caused by the materials in the kairomonal bait. Buffalo gourd root powder contains cucurbitacins E and I. In laboratory bioassays, 0.03 μg of cucurbitacin E elicited a feeding response in *D. u. howardii*, but 10 μg, an increase of three orders of magnitude, was required to elicit feeding by *A. vittata* (response to cucurbitacin I was not reported) (Metcalf & Lampman 1989). Both species, however, are sensitive to the carbaryl toxicant (topical LD_{50} against *A. vittata* and *D. u. howardii* of 0.0081 and 0.11 μg per beetle, respectively; J. Gaggero, personal comm). The lower sensitivity to the phagostimulant by *A. vittata* may explain the increased plant feeding by this species relative to other Diabroticites. Meanwhile, the small amount needed to achieve a lethal dose may result in high mortality rates. Also, no effort was made to optimize field application rates. For cucurbit crops, it may be possible to optimize the product (both cucurbitacins and nonpheromonal volatiles) and rate with respect to the behavior of *A. vittata* and *D. u. howardii*.

A second hypothesis relates to host preferences. *D. u. howardii* is considered to have a relatively wide host range, and the other two *Diabrotica* spp. probably eclosed in a cornfield and fed on corn (possibly silks) before immigrating into the treated plots. In contrast, *A. vittata* may be more of a specialist on cucurbits and may have sought out and fed on other cucurbits in the landscape prior to immigrating into the treated plots. Although most cultivated cucurbits have relatively small amounts of cucurbitacins, this specialist behavior may have resulted in prior exposure to cucurbitacins. Prior exposure has been shown to reduce sensitivity to cucurbitacins (Tallamy & Halaweish 1993).

Sex may also be an important factor influencing the impact of the bait on feeding incidence and intensity. Although sex was not a significant component of the categorical models for the caged data, it was a significant explanatory variable for feeding incidence in *D. u. howardii* and *D. v. virgifera*, and also for survivorship in *D. v. virgifera*, under open plot conditions (Table 5). Under field conditions in both of these species, there was a sexual bias for males to feed on plants rather than, or in addition to, the bait. Under laboratory conditions, reproductive activity depressed sensitivity to cucurbitacins in males, but increased sensitivity in females (Tallamy & Halaweish 1993). Depressed sensitivity to cucurbitacins would be expected to reduce the

impact of the bait, resulting in greater feeding on plants. Thus, the field data may reflect reproductive activity in the population of these two species. For live *D. v. virgifera,* there was also a greater tendency to capture males regardless of kairomonal treatment (Table 5). This may reflect sexual differences in movement behavior between corn and cucurbits, potentially being influenced by the nonpheromonal volatile components in the bait. However, reproductive status and other biological parameters that may help explain variation in response were not measured, and further field work is warranted.

Regardless of the mechanism, the increased probability of acquiring a mark by *A. vittata* has implications about the relative impact of these four species on bacterial wilt epidemiology and on the potential of behavior modification with semiochemicals to influence transmission of *E. tracheiphila* in cucurbits. The kairomonal baits dramatically reduced survivorship of all four species and, thus, can also be expected to reduce pathogen transmission. This would occur by reducing the time that vectors are alive on the crop, as in most contact insecticides. However, kairomonal baits offer the additional potential of blocking trophic interactions with the plants during the time, however brief, that the insect is alive and feeding on the crop. In these field and caged trials, this potential was demonstrated for three *Diabrotica* species, but not for *A. vittata* in the field studies, and less so for *A. vittata* than *D. u. howardii* in the cage studies. This reduction of trophic interaction was greater for females than males in two species (*D. u. howardii* and *D. v. virgifera*). Currently, there is little known about bacterial wilt epidemiology and the relative biological capability of pathogen transmission among species and sex. These studies develop novel methods, and demonstrate that kairomonal baits affect Diabroticite trophic interactions and may reduce pathogen transmission, but that complex behavioral interactions causes variation in this effect among species and sex.

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References Cited

Adams, R. G., R. A. Ashley & M. J. Brennan. 1990. Row covers for excluding insect pests from broccoli and summer squash plantings. J. Econ. Entomol. 83: 948–954.

Akey, D. H., J. L. Hayes & S. J. Fleischer. 1991. Use of elemental markers in the study of arthropod movement and trophic interactions. Southwest. Entomol. Suppl. 14: 87.

Alverson, D. R., J. N. All & P. B. Bush. 1980a. Rubidium as a marker and simulated inoculum for the black-faced leafhopper, *Graminella nigrifons,* the primary vector of maize chlorotic dwarf virus of corn. Environ. Entomol. 9: 29–31.

Alverson, D. R., J. N. All & C. W. Kuhn. 1980b. Simulated intrafield dispersal of maize chlorotic dwarf virus by *Graminella nigrifrons* with a rubidium marker. Phytopathology 70: 734–737.

Andersen, J. F. & R. L. Metcalf. 1986. Identification of a volatile attractant for *Diabrotica* and *Acalymma* species from the blossoms of *Cucurbita maxima* Duchesne. J. Chem. Ecol. 12: 687–699.

1987. Factors influencing the distribution of *Diabrotica* spp. in the blossoms of cultivated Cucurbita spp. J. Chem. Ecol. 14: 681–689.

Brewer, M. J., R. N. Story & V. L. Wright. 1987. Development of summer squash seedlings damaged by striped and spotted cucumber beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 80: 1004–1009.

Buhler, W. G., A. C. York & R. F. Turco. 1992. Effect of enhanced biodegradation of carbofuran on the control of striped cucumber beetle (Coleoptera: Chrysomelidae) on muskmelon. J. Econ. Entomol. 85: 1910–1918.

Culin, J. D. & D. R. Alverson. 1986. A technique to mark adult *Heliothis zea* using rubidium chloride-spiked artificial nectar sources. J. Agric. Entomol. 3: 56–60.

Ferguson, J. E., E. R. Metcalf, R. L. Metcalf & A. M. Rhodes. 1983. Influence of cucurbitacin content in cotyledons of cucurbitaceae cultivars upon feeding behavior of Diabroticine beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 76: 47–51.

Fleischer, S. J., M. J. Gaylor & N. V. Hue. 1988. Dispersal of *Lygus lineolaris* (Heteroptera: Miridae) adults through cotton following nursery host destruction. Environ. Entomol. 17: 533–541.

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

Fleischer, S. J., F. W. Ravlin, R. J. Stipes & M. C. Grender. 1989. Incorporation of rubidium into pin oak and gypsy moth (Lepidoptera: Lymantridae): marking a tree defoliator for movement studies. Ann. Entomol. Soc. Am. 82: 686–692.

Freeman, D. H. 1987. Applied categorical data analysis. Marcel Decker, New York.

Graham, H. M. & D. A. Wolfenbarger. 1977. Tobacco budworm: labelling with rubidium in the laboratory. J. Econ. Entomol. 70: 800–802.

Knight, A. L., L. A. Hull, E. G. Rajotte & S. J. Fleischer. 1989. Labelling tufted apple budmoth (Lepidoptera: Tortricidae) with rubidium: effect on development, longevity, and fecundity. Ann. Entomol. Soc. Am. 82: 481–485.

Lampman, R. L. & R. L. Metcalf. 1987. Multicomponent kairomonal lures for southern and western

corn rootworms (Coleoptera: Chrysomelidae *Diabrotica* spp.) J. Econ. Entomol. 80: 1137–1142.

1988. The comparative response of *Diabrotica* species (Coleoptera: Chrysomelidae) to volatile attractants. Environ. Entomol. 17: 644–648.

Lance, D. R. & G. R. Sutter. 1991. Semiochemical-based toxic baits for *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae): effects of particle size, location, and attractant content. J. Econ. Entomol. 84: 1861–1868.

Legg, D. E. & H. C. Chiang. 1984. Rubidium marking technique for the European corn borer. Environ. Entomol. 13: 579–583.

Lewis, P. A., R. L. Lampman & R. L. Metcalf. 1990. Kairomonal attractants for *Acalymma vittatum* (Coleoptera: Chrysomelidae). Environ. Entomol. 19: 8–14.

Metcalf, R. L. 1985. Plant kairomones and insect pest control. Ill. Nat. Hist. Surv. Bull. 33: 175–198.

Metcalf, R. L. & R. L. Lampman. 1989. The chemical ecology of Diabroticites and Cucurbitaceae. Experimentia. 45: 240–247.

Metcalf, R. L., J. E. Ferguson, R. Lampman & J. F. Andersen. 1987. Dry cucurbitacin-containing baits for controlling Diabroticite beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 80: 870–875.

Metcalf, R. L., R. A. Metcalf & A. M. Rhodes. 1980. Cucurbitacins as kairomones for diabroticite beetles. Proc. Natl. Acad. Sci. 77: 3769–3772.

Metcalf, R. L., A. M. Rhodes, R. A. Metcalf, J. Ferguson, E. R. Metcalf & P. Lu. 1982. Cucurbitacin contents and Diabroticite (Coleoptera: Chrysomelidae) feeding upon *Cucurbita* spp. Environ. Entomol. 11: 931–937.

Metcalf, R. L. & E. R. Metcalf. 1992. Plant kairomones in insect ecology and control. Cheyemen & Hall, New York.

Rand, F. V. & E.M.A. Enlows. 1920. Bacterial wilt of cucurbits. U.S. Dep. Agric. Bull. 828.

SAS Institute. 1989. SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute, Cary, NC.

Shepard, M. & V. H. Waddill. 1976. Rubidium as a marker for Mexican bean beetles, Epilachnia varivestis. Can. Entomol. 108: 337–339.

Sherf, A. F. & A. A. MacNab. 1986. Vegetable Diseases and Their Control. Wiley, New York.

Stimmann, M. W. 1974. Marking insects with rubidium: imported cabbageworm marked in the field. Environ. Entomol. 3: 327–328.

Stimmann, M. W., W. W. Wolf & W. L. Berry. 1973. Cabbage loopers: biological effects of rubidium in the larval diet. J. Econ. Entomol. 66: 324–326.

Tallamy, D. G. 1985. Squash beetle feeding behavior: an adaptation against cucurbit defenses. Ecology 66: 1574–1579.

Tallamy, D. G. & F. T. Halaweish. 1993. Effects of age, reproductive activity, sex, and prior exposure on sensitivity to cucurbitacins in southern corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 22: 925–932.

Tallamy, D. G. & V. A. Krischik. 1989. Variation and function of cucurbitacins in *Cucurbita:* an examination of current hypotheses. Am. Nat. 133: 766–786.

Van Steenwyk, R. A., G. R. Ballmer, A. L. Page, T. J. Ganje & H. T. Reynolds. 1978a. Dispersal of rubidium-marked pink bollworm. Environ. Entomol. 7: 608–613.

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

Voss, R. H. & D. N. Ferro. 1985. Labelling the Colorado potato beetle with rubidium to study colonization and dispersal processes, pp. 53–62. *In* D. N. Ferro & R. H. Voss [eds.], Proceedings, Symposium on the Colorado Potato Beetle, XVIIth International Congress of Entomology. Mass. Agr. Exp. Stn. Res. Bull. 704.

Weissling, T. J., L. J. Meinke & K. A. Lytle. 1991. Effect of starch-based corn rootworm (Coleoptera: Chrysomelidae) baits on selected nontarget insect species: influence of semiochemical composition. J. Econ. Entomol. 84: 1235–1241.

Wolfenbarger, D. A., H. M. Graham, J. B. Nosky & O. H. Lindig. 1982. Boll weevil (Coleoptera: Curculionidae): marking with rubidium chloride sprays on cotton and dispersal from cotton. J. Econ. Entomol. 75: 1038–1041.

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