An experiment using neutron activation analysis and a rare earth element to mark cotton plants and two insects that feed on them

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Abstract

Studies on insect dispersal and other behaviors can benefit from using markers that will not alter flight and fitness. Rare earth elements, such as samarium (Sm), have been used as ingested markers of some insects and detected using neutron activation analysis (NAA). In this study, samarium nitrate hexahydrate was mixed into artificial diet for boll weevils, Anthonomus grandis grandis Boheman (Coleoptera: Curculionidae), at different dosages and in water used to irrigate cotton, Gossypium hirsutum L. Samarium was detected in adult boll weevils fed on the samarium-labeled diet, but not after 5 or 10 days of being switched to non-labeled diet, even if the insects were given labeled diet for as long as 7 consecutive days. Introduced in irrigation water, 1% samarium (m/m) was detectable in cotton squares and leaf tissue. However, boll weevil adults fed samarium-labeled squares did not retain detectable levels of samarium, nor did boll weevil adults reared to adulthood from samarium-labeled squares. Fourth instar beet armyworms, Spodoptera exigua (Huëbler) (Noctuidae: Lepidoptera), fed on samarium-labeled cotton leaves obtained enough samarium for NAA detection, but adult moths reared from them did not have detectable amounts of samarium. Although samarium can be useful as a marker when insects are presented with a continuous pulse of the label, elements that are assimilated by the insect would be more useful if a continuous infusion of the marker cannot be provided.

1. Introduction

To study insect behaviors, such as dispersal, effective markers should not affect the flight and fitness of the insect, and should not hinder, irritate, or change the insect’s behavior, growth, reproduction, or life span (Southwood, 1966; Showler et al., 1988; Hagler and Jackson, 2001; Showler, 2004). Delivery of the markers through labeled food, usually living plants or artificial diet, is least intrusive (Showler et al., 1989; Qureshi et al., 2004a, b). Although radiotracers are arguably more versatile for use in insect ecology and ethology than other markers and can be detected with higher sensitivity (Showler et al., 1988, 1990), public concern regarding the potential detrimental effects of ionizing radiation has limited their application. Some researchers have turned to neutron activation analysis (NAA) to avoid the introduction of radioactivity into the environment (Jenkins, 1963; Monro, 1968; Costa and Byrne, 1988; Showler et al., 1990). NAA methods provide radiation safety in the environment, a diversity of nuclides from which to choose, and sensitivity of detection (Showler et al., 1988). The technique also permits long-term experiments without decay of the tracer, non-destructive sample analysis (the sample insect is killed, but not destroyed, and can be analyzed by NAA repetitively), and quantification of the label in each sample based upon the amount of radiation emitted (Wang et al., 1975; Knaus and Curry, 1979; Showler et al., 1989). The activated nuclide can be detected and quantified using the...
methods available for conventional radiotracers (Showler et al., 1988).

While toxicity to the insect might result from the nuclides that can be activated by neutrons, detectable non-lethal levels can be obtained by experimentation (Curtis et al., 1973). Application of these nuclides to the insect has been achieved through ingestion (Richardson, 1969; Richardson et al. 1971; Curtis et al., 1973; Showler et al., 1989; Weeks et al., 2004) and by absorption of cerium into the cuticle of palm weevils, Rhyncocophorus ferrugineus Olivier (Rahalkar et al., 1971).

Mustard, Brassica hirta Moench, maize, Zea mays L., and hairy vetch, Vicia villosa Roth, have been shown to translocate dysprosium and europium from soil to leaves (Kloke and Riebartsch, 1965; Xu et al., 2003). Thus, certain herbivorous insects might be labeled from feeding on tagged host plants. By extension, food webs could be examined using NAA techniques (Zhang et al., 2000), and insect populations feeding in a field of marked plants could conceivably take on the label. Radiotracers can be transferred between insect development stages, including species that undergo complete metamorphosis (Kettlewell, 1952; Yagi, 1958; Mayer and Brazzel, 1961), but this has been demonstrated using stable-activable technology only once with cesium (not a rare earth element) in southwestern corn borers, Diatraea grandiosella Dyar (Qureshi et al., 2004a, b). The purpose of this study was to determine the extent to which a rare earth element, samarium, can be used to label cotton plants and two insect herbivores that feed on different parts of the plant.

2. Materials and methods

Cotton, Gossypium hirsutum L., var. ‘DP5415RR’, was grown in this study in 7.6-l pots with Sunshine No. 1 nursery potting soil (∼75% sphagnum peat moss, perlite, dolomitic limestone, and gypsum; Sungro Horticulture, Bellevue, Washington) kept in a greenhouse at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center (KSARC) in Weslaco, Texas. Seedlings were thinned to two per pot, and 200 ml of Peters Professional (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) water-soluble general-purpose 20–20–20 N–P–K fertilizer at 15.8 g/l of water was applied to each pot at the two true leaf stage. The pots were irrigated with 1 l of water containing 1% samarium nitrate (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) water-soluble general-purpose 20–20–20 N–P–K fertilizer at 15.8 g/l of water was applied to each pot at the two true leaf stage. The pots were irrigated with 1 l of water two times each week, all the pots were placed in a 1 × 1 × 1-m cage in a greenhouse with one pot of cotton plants, and this was replicated five times. A control, whereby the irrigation water had no samarium, was also replicated five times. The pots of plants were removed after two days, and squares that abscised because of oviposition, boll weevil larvae developing inside (Showler and Cantú, 2005), were collected and placed in separate Petri dishes stored in an environmental chamber at 26.7°C and 35% RH. Emerging adult boll weevils were killed by freezing and stored at −80°C until NAA.

In a separate assay, pots of cotton plants were irrigated with 11 of water containing 1% samarium nitrate hexahydrate. After 2 days, five mated female boll weevils were placed in a 1 × 1 × 1-m cage in a greenhouse with one pot of cotton plants, and this was replicated five times. A control, whereby the irrigation water had no samarium, was also replicated five times. The pots of plants were removed after two days, and squares that abscised because of oviposition, boll weevil larvae developing inside (Showler and Cantú, 2005), were collected and placed in separate Petri dishes stored in an environmental chamber at 26.7°C and 35% RH. Emerging adult boll weevils were killed by freezing and stored at −80°C until five weevils were selected at random for NAA.

2.1. Samarium-labeled boll weevil diet

Samarium nitrate hexahydrate was homogenized with artificial boll weevil diet (Stewart et al., 1972) at 0.01%, 0.1%, and 1% (m/m). Newly emerged adult boll weevils (sex ratio was not determined) were fed one of the three samarium-labeled diet treatments for two and seven consecutive days, or a non-labeled (control) diet. After the feeding period, five weevils from each treatment—feeding period combination were removed and killed by freezing at −80°C, or allowed to feed on two fresh, non-labeled cotton squares (4–8 mm in diameter), replaced daily, for 5 or 10 days, then frozen at −80°C. The control weevils were fed squares for 10 days and frozen.

2.2. Samarium labeling cotton squares and leaves

Samarium nitrate hexahydrate was mixed with water at 0.01%, 0.1%, and 1% (m/m) using a vortex magnet stirring bar in a 100-ml beaker for 1 h. After one irrigation was skipped, a liter of each mixture or non-labeled control water was poured into five pots of cotton plants. Two days later, one leaf from the top three fully expanded apical leaves and one 5–6-mm-diameter square were removed from a plant in each pot and stored at −80°C until NAA.

2.3. Feeding boll weevils samarium-labeled squares

Adult boll weevils were placed for 2 days in separate Petri dishes with two 5-7-mm-diameter samarium-labeled cotton squares from plants irrigated with 0, 0.01, 0.1, or 1% samarium nitrate hexahydrate (five replications). The weevils were killed and stored at −80°C until NAA.

In a separate assay, pots of cotton plants were irrigated with 11 of water containing 1% samarium nitrate hexahydrate. After 2 days, five mated female boll weevils were placed in a 1 × 1 × 1-m cage in a greenhouse with one pot of cotton plants, and this was replicated five times. A control, whereby the irrigation water had no samarium, was also replicated five times. The pots of plants were removed after two days, and squares that abscised because of oviposition, boll weevil larvae developing inside (Showler and Cantú, 2005), were collected and placed in separate Petri dishes stored in an environmental chamber at 26.7°C and 35% RH. Emerging adult boll weevils were killed by freezing and stored at −80°C until five weevils were selected at random for NAA.

2.4. Feeding beet armyworm larvae samarium-labeled leaves

Five pots of cotton plants were irrigated with 11 of 1% samarium nitrate hexahydrate, and five other pots (controls) were irrigated with non-labeled water. After two days, five fourth instar beet armyworms were placed on the
upper fully expanded leaves of each plant and allowed to feed for 2 days. Five randomly selected larvae were removed from the samarium treatment and from the control, then killed by freezing and stored at −80 °C until NAA. Pupae from the other larvae that remained on the plants were stored in separate Petri dishes with vermiculite until adult moths emerged. Five moths that developed from larvae fed on samarium-labeled leaves and from control plants were killed upon emergence from pupae by freezing and stored at −80 °C until NAA.

2.5. Neutron activation analysis

The NAA protocol followed in this work that used earlier for studies on the fire ant (Showler et al., 1989; Weeks et al., 2004). Samples were encapsulated in pre-cleaned polyethylene irradiation vials. Comparator laboratory reference samples (Zeev and Alfassi, 1990) were prepared by evaporation of weighed quantities of primary samarium laboratory reference sample solutions onto high-purity cellulose powder. Laboratory reference samples were prepared at levels ranging from 1 to 100 ng Sm. The mass of cellulose was varied to allow matching of volumes with analyzed samples to minimize uncertainties due to sample/standard geometry differences. Reference Material SRM1571, orchard leaves, with a known samarium content of 114 ± 20 ng/g was included with each batch of eight weevil, cotton plant, or beet armyworm samples to provide assurance of accuracy. Samples and laboratory reference samples were then irradiated together in the Texas A&M Nuclear Science Center’s 1 MW research reactor facility for 14 h at a nominal neutron flux of 1 × 1013 cm−2 s−1 in a rotisserie (rotating) position. All irradiated materials underwent gamma-ray spectrometry 2–6 days later, depending on the sample matrix. The distance from the sample to the detector was varied depending on the sample matrix. Spectra were accumulated for 1 h on a Canberra Industries (Meriden, CT) Alpha-based VMS Genie system using high-purity germanium detectors. The resolution of the used detectors was high (better than 1.70 keV full width at half maximum for the 1332 keV line of 60Co). The 103-keV gamma line from 153Sm was utilized for computation of samarium content with Canberra’s Genie NAA software. Decay periods before the analyses were adjusted so that the 103-keV line was optimally resolved in the complex spectra obtained. Detection limits were 36 pg Sm for weevils, 122 pg Sm for cotton squares, and 117 pg Sm for leaves. These detection limits were calculated by the well-established algorithm (Curie, 1968), which is incorporated into the Canberra spectroscopy software. Under the condition of “well-known blank” (the spectral background in the peak location) and a confidence level ±β = 0.05, the working expression for the determination level in units of counts is \( L_D = 2.71 + 3.29\sigma_a \). The sensitivity was readily sufficient to measure samarium in control insects down to 220 pg and to monitor the assimilation of as little as 1 ng of the element in boll weevils. Results from the repeated analyses \( n = 21 \) of the QC material SRM of 111.5 ± 6.3 ng/g compared well with the certified value of 114 ± 2 ng/g.

2.6. Statistical analysis

Assays with more than one treatment (including the control) were analyzed as completely randomized designs using one-way ANOVA, and means were separated using Tukey’s HSD (Analytical Software, 1998). Assays with one treatment and a control were analyzed with the two-sample t-test (Analytical Software, 1998).

3. Results

3.1. Samarium-labeled boll weevil diet

NAA detected samarium in levels that were distinguishable from the control \( P < 0.05 \). Boll weevils that fed on the samarium-labeled artificial diet for 2 or 7 days were marked with detectable amounts of samarium (a sample spectrum is shown in Fig. 1), but only when the weevils were frozen immediately after being removed from the labeled diet \( F(2,20) = 22.98; \text{df} = 18, 94; P < 0.0001 \) (Table 1). Weevils that fed on non-labeled squares for 5 or 10 days after removal from the artificial diet did not contain detectable amounts of samarium (Table 1). Amounts of samarium in weevils fed for 2 days on 0.1% and 1% samarium-labeled diets were 4.6 and 7.4 times greater, respectively, than in weevils fed on the 0.01% samarium-labeled diet. The amounts of samarium in weevils that were on samarium-labeled diet for 7 days were statistically the same as in the insects fed for 2 days (Table 1). As an example, at the 0.1% dosage, weevils fed samarium-labeled diet for 7 days had 84% less samarium \( P < 0.05 \) than weevils fed for 2 days (Table 1). However, the greatest average amount of samarium, 182.7 ± 39.6 ng, was detected in weevils fed 1% samarium-labeled diet for 7 days.

![Fig. 1. A gamma spectrum of a neutron-activated 2.2-mg boll weevil. The determined amount of Sm is 70 pg (30 ppb). The inset shows a magnified portion of the spectrum in the vicinity of the Sm line.](image-url)
Table 1
Amounts of samarium in adult boll weevils fed for 0, 2, or 7 days on artificial diet labeled with 0, 0.01, 0.1, or 1% (m/m) samarium nitrate hexahydrate after 0, 5, or 10 days of being replaced with non-labeled diet (n = 5)

<table>
<thead>
<tr>
<th>Concentration of the marker in the diet (%)</th>
<th>No. of days fed</th>
<th>No. of days post-feeding</th>
<th>Amount of Sm in weevil(a) (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>0 e</td>
</tr>
<tr>
<td>0.01</td>
<td>2</td>
<td>0</td>
<td>13.8 ± 4.4 b c</td>
</tr>
<tr>
<td>0.01</td>
<td>7</td>
<td>0</td>
<td>14.0 ± 9.8 c d</td>
</tr>
<tr>
<td>0.10</td>
<td>2</td>
<td>0</td>
<td>77.7 ± 27.8 ab</td>
</tr>
<tr>
<td>0.10</td>
<td>7</td>
<td>0</td>
<td>10.3 ± 4.4 cd</td>
</tr>
<tr>
<td>1.00</td>
<td>2</td>
<td>0</td>
<td>102.6 ± 22.4 a</td>
</tr>
<tr>
<td>1.00</td>
<td>7</td>
<td>0</td>
<td>182.7 ± 40.0 a</td>
</tr>
<tr>
<td>0.01</td>
<td>2</td>
<td>5</td>
<td>0.1 ± 0.1 e</td>
</tr>
<tr>
<td>0.01</td>
<td>7</td>
<td>5</td>
<td>0.2 ± 0.1 e</td>
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<td>0.10</td>
<td>2</td>
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<td>0.10</td>
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<td>1.00</td>
<td>2</td>
<td>5</td>
<td>0.6 ± 0.2 de</td>
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<tr>
<td>1.00</td>
<td>7</td>
<td>5</td>
<td>0.7 ± 0.2 de</td>
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<tr>
<td>0.01</td>
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<td>0.01</td>
<td>7</td>
<td>10</td>
<td>0.1 ± 0.1 e</td>
</tr>
<tr>
<td>0.10</td>
<td>2</td>
<td>10</td>
<td>2.9 ± 2.4 de</td>
</tr>
<tr>
<td>0.10</td>
<td>7</td>
<td>10</td>
<td>0.4 ± 0.1 de</td>
</tr>
<tr>
<td>1.00</td>
<td>2</td>
<td>10</td>
<td>0.5 ± 0.2 de</td>
</tr>
<tr>
<td>1.00</td>
<td>7</td>
<td>10</td>
<td>1.0 ± 0.1 de</td>
</tr>
</tbody>
</table>

Values followed by the same letter are not significantly different (P > 0.05). The 1% treatment resulted in 12.1 times more samarium than the 0.1% treatment (Table 2).

Samarium was detected in leaf tissue samples from plants irrigated with 0.1% and 1% samarium-labeled water (F = 30.69; df = 3, 19; P < 0.0001). The 0.1% samarium treatment was 12.5 times greater (P < 0.05) in abundance of samarium than the 0.01% treatment, and leaf tissue from the 1% treatment had 13.3 times more samarium (P < 0.05) than the 0.1% treatment (Table 2). Leaf and square tissues within each treatment had the same concentrations of samarium.

3.3. Feeding boll weevils samarium-labeled squares

Adult boll weevils that fed on cotton squares labeled through irrigation with 0.01%, 0.1%, or 1% samarium-labeled water did not have detectable levels of samarium. Similarly, boll weevils reared on squares labeled by irrigating cotton plants with 1% samarium-tagged water were not marked by detectable quantities of samarium.

3.4. Feeding larval beet armyworms samarium-labeled leaves

Fourth instar beet armyworms fed on samarium-labeled leaves had approximately 12.5 ng samarium, more than the control (t = 2.47; df = 1, 8; P = 0.0389) (Table 2), but adult moths developed from labeled larvae did not have detectable quantities of samarium.

4. Discussion

Samarium was chosen for use in these experiments because the sensitivity of its detection by NAA is high. The choice of NAA rather than atomic spectroscopic methods is based on this high sensitivity, which is due, in part, to the relatively large cross-section of 152Sm (over 200 b). Our determination limit was computed as approximately 36 pg, which compares favorably with another estimate (50 pg) for this method (Morrison, 1965). Estimates of detection limits by other methods include 1000 ng (Morrison, 1965) and 500 ng/ml (Dean, 1995) for AAS, and approximately 100 ng/ml for ICP-OES (Dean, 1995).

Curculionids and noctuids have been tagged using visual markers (Gast and Landin, 1966; Southwood, 1966; Lloyd et al., 1968; Daum et al., 1969), rubidium (Graham and Wolfenbarger, 1977; Graham et al., 1978; Wolfenbarger et al., 1982; Jost and Pitre, 2002), and radiotracers (Mayer and Brazzel, 1961; Hines et al., 1973; Moore et al., 1974). Palm weevils were marked with cesium, which is not a rare earth element, through absorption into the cuticle (Rahalkar et al., 1971). In studies on imported fire ants, Solenopsis invicta Buren, samarium was spread through colonies by trophallaxis (Howard and Tschinkel, 1981; Sorenson et al., 1985) in order to study movement of food through colonies (Weeks et al., 2004) and territorial
behavior of colonies in an agricultural system (Showler et al., 1989).

Our study demonstrated that, although ingested, samarium was apparently excreted within 5 days and, hence, made for a poor marker unless the weevils were given a continuous supply of samarium-containing food, as were the imported fire ants in other studies (Showler et al., 1989; Weeks et al., 2004). Given a continuous supply of the labeled artificial diet, boll weevils ingested samarium generally corresponding to the dosage, hence the 1% dosage resulted in the most samarium detected in the weevils.

Radio-tracers become incorporated into the insect body if they are biologically essential elements or mimics of biologically essential elements (Showler et al., 1988). As an example, an imported fire ant colony remained radioactive due to $^{65}$Zn 6 months after the radio-labeled molasses bait was removed (Showler et al., 1990). Rare earth elements are not biologically essential, and they do not mimic biologically essential elements. However, some non-rare earth’s elements, like cesium, do mimic essential elements, such as potassium (Moss and Van Steenwyk, 1982; Jost and Pitre, 2002). They can be used in NAA and hence might make better markers than rare earth elements for single pulse deliveries. Without a continuous pulse delivery system for a rare earth element, such as samarium, boll weevils might retain cesium or other assimilated marker elements longer without the labeled bait available.

Plants are known to translocate rare earth elements (Wang et al., 2001; Hu et al., 2004), and our study revealed that cotton plants can take up samarium from irrigation water and translocate it to both leaves and fruit. Further experimentation, however, will be required to determine the duration that samarium is retained in the plant after a single-pulse application of the label and the extent to which bioaccumulation might occur (Wang et al., 2003; Xu et al., 2003).

The translocation of samarium to parts of the cotton plants that are commonly consumed by important pests can allow for delivery through the ecosystem and sensitive detection (Showler et al., 1988). Delivery of sensitive markers from labeled plants to herbivorous insects has been accomplished with radio-tracers (Yagi, 1958; Krall and Simmons, 1977), and in some studies the radiotracer was detected in the predatory arthropods that consumed the herbivore on its radio-labeled host plant (Pendleton and Grundmann, 1954; Odum and Kuenzler, 1961; McCarty et al., 1980). This is the first study to examine the potential use of a rare earth element to track herbivorous insects through feeding on systemically labeled host plants. Our study demonstrated that samarium can be obtained from labeled host plants by beet armyworm larvae, but not boll weevils, presumably because of the quantities to be found passing through the gut at any given time. We conjecture that the gut of a fourth instar beet armyworm holds more samarium-labeled plant tissue than that of an adult boll weevil. Thus, a continuous pulse of samarium in artificial diet, and in leaf tissue, might result in labeling of adult boll weevils and larval beet armyworms, respectively.

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