Rubidium Marking to Detect Dispersal of Pest and Predator from Corn into Sorghum and Cotton in Georgia

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Abstract

This field study evaluated marking both the pest [Helicoverpa zea (Boddie)] and its predator [Orius insidiosus (Say)] with rubidium chloride (RbCl) in corn to detect dispersal of these insects from this crop into sorghum and cotton. Sorghum and cotton were planted in small plots at the interface, or common boundary, of a commercial corn and cotton field. The cotton field adjacent to these interface plots was divided into cotton field plots. Foliar solutions of RbCl were applied to corn at site 1 when the ears were infested with 4th through 6th instars of H. zea and at site 2 when corn silks were infested with eggs and 1st instars of H. zea and nymphs and adults of O. insidiosus. Insects were collected at various times after RbCl application from the sorghum interface plots, cotton interface plots, and cotton field plots. Both H. zea eggs and O. insidiosus females were successfully marked in corn treated with RbCl, and marking success for both insects ranged from 15-33%. Data on rubidium-marked insects indicated that H. zea females from the generation feeding on rubidium-treated corn dispersed into sorghum interface plots at both treated sites and into cotton interface plots at one site and that O. insidiosus females dispersed from corn into sorghum interface plots and cotton field plots at one site.

Key Words

dispersal, Helicoverpa zea, Orius insidiosus

Populations of the corn earworm, Helicoverpa zea (Boddie), buildup early-season in corn and can occur mid to late season on cotton and sorghum (Quaintance and Brues 1905). Orius insidiosus (Say) is an important predator of eggs and young larvae of this pest (Barber 1936). Both H. zea females and O. insidiosus adults are more attracted to corn in the silking stage than in any other developmental stage of this plant, and thus both insect species are abundant at the same time on this crop (Barber 1936). Similarly, in sorghum, adults of both the pest and predator are attracted to flowering panicles, and thus the appearance of O. insidiosus closely coincides with the presence of eggs of its prey, the corn earworm (Tillman 2006). The question is do both of these insect species disperse from corn into sorghum and cotton?

The method of elemental marking of biological systems using rubidium, proposed by Berry et al. (1972), is a powerful and versatile technique for detecting dispersal of

Applying RbCl to plants in the field or to diet in the laboratory has been used successfully to mark several pests, including the tobacco budworm, Heliothis virescens (F.) (Graham and Wolfenbarger 1977), the beet armyworm, Spodoptera exigua (Hübner) (Pearson et al. 1989), the fall armyworm, Spodoptera frugiperda (J. E. Smith) (Graham et al. 1978a), the blueberry leafhopper, Groesia curvulana (Kearfoot) (Polavarapu et al. 1992), the potato tuberworm, Phthorimaea operculella (Zeller) (Coll et al. 1997), and the Mexican bean beetle, Epilachna varivestis Mulsant (Shepard and Waddill 1976). Graham et al. (1978a) conducted one of the first experiments to assess the potential of using rubidium to mark *H. zea*. In their field experiments, *H. zea* larvae on RbCl-treated sorghum and cotton showed elevated levels of rubidium compared with larvae feeding on untreated plants. In later field experiments, foliar applications of RbCl were used to effectively mark *H. zea* adults that developed on corn (Graham et al. 1978b) and wild geranium (Stadelbacher 1991).

Natural enemies of insect pests are marked through consumption of marked herbivores (Graham et al. 1978a, 1978b, Johnson and Reeves 1995) or possibly through feeding on rubidium-treated plants or pollen. Both predators and parasitoids of *H. zea* have been successfully marked with rubidium. The parasitoid, Trichogramma pretiosum Riley, and the predator, Chrysoperla rufilabris Burmeister, were effectively marked with rubidium in the laboratory (Shaver et al. 1990). The predator, Geocoris punctipes (Say), also was labeled with rubidium through feeding on Rb-treated artificial diet (Cohen and Jackson 1989). Eight different predator taxa, including Hippodamia convergens Guérin-Méneville, O. insidiosus, assorted Araneae, Scymnus loewii Mulsant, Nabis spp., Geocoris spp., Notoxus sp., and Colllops spp., were successfully marked in sorghum and cotton field plots (Prasifka et al. 2001).

This field study was conducted to determine if marking both the pest *H. zea* and its predator *O. insidiosus* with rubidium in corn could be used to detect dispersal of these 2 species of insects from corn into sorghum and cotton.

**Materials and Methods**

**Sampling design.** This field study was conducted in Irwin Co., GA, in 2002. Three plots (45 m wide x 12 rows deep) of sorghum and 3 equally-sized plots of cotton were planted in a strip at the interface, or common boundary, of a commercial corn and cotton field (Fig. 1). Each interface plot was subdivided into three 15 m long sections. In the sorghum interface plots, panicles from 3 sorghum plants were randomly obtained per section per row so that 108 (3 sections x 4 rows x 3 samples x 3 plots) panicles were collected per site. All rows in which sorghum panicles were available were sampled. In cotton interface plots, 6 cotton plants per section were sampled per plot for 54 (3 sections x 6 samples x 3 plots) plants sampled per site. The cotton field adjacent to the interface plots was divided into 6 cotton field plots (45 m wide x 180 m deep). Each cotton field plot was associated with either a sorghum interface plot or a cotton interface plot as described above. Each cotton field plot was subdivided into three 15 m sections. The cotton field plots were further subdivided in 15 x 15 m blocks.
Fig. 1. Diagrammatic representation of experimental plots for rubidium study (not drawn to scale). Each cotton (COT) and sorghum (SOR) interface plot was subdivided into three 15-m long sections. The cotton field adjacent to the cotton and sorghum interface plots was divided into six field cotton plots so that each interface plot was associated with a field cotton plot. In the cotton field, each block refers to a 15 by 15 m area in which a random sample was obtained. The number represents the greatest distance (m) in the block away from the interface plots, i.e. 15 means 1-15 m away from the interface plots.

representing the distance away from the interface plots. A single plant was randomly sampled in each 1-15 m, 16-30 m, 46-60 m, 91-120 m, and 151-180 m block for all three sections and in each 31-45 m, 61-90 m, and 121-150 m block for only the center section for a total of 108 (18 plants per plot x 6 plots) plants sampled per site. This arrangement of interface plots and cotton field plots was repeated at three sites. At two of these sites, the corn was treated with rubidium chloride (RbCl). The other site was used as an untreated control. This control field site was ~10 km away from the two treated sites.

The commercial cotton fields ranged from 8-10 ha, and the commercial corn fields associated with these cotton fields ranged from 8-12 ha. All cotton (Delta Pine 5,690 Roundup Ready) was planted on 10 May. All corn (P31G98) was planted the first week of April. Sorghum (DeKalb E57) was planted a rate of 25,500 seeds/ha using a 2-row John Deere (Deere & Co., Moline, IL) planter on three planting dates, 13 May, 23 May, and 3 June. On each planting date, sorghum was planted in 4 rows with the first planting placed adjacent to the corn field.

Rubidium application. RbCl solutions were prepared using solid RbCl (Sigma, St. Louis, MO) at a minimum of 99% purity. The solution (10 g RbCl/L of water) was applied to corn plants using a hand-held sprayer, for foliar sprays of RbCl dissolved
in water are favored for field applications because of overall effectiveness and the accuracy of directed sprays for marking defined areas (Guillebeau et al. 1993). The first 10 rows of corn along the length of the common boundary of the corn and cotton field were treated. Only ears of corn were treated to direct the application of the RbCl solution to the site of insect feeding.

At field site 1, RbCl was applied to the ears of corn on 18 June when the ears were infested with 4th, 5th, and 6th instars of H. zea. At field site 2, RbCl was applied to ears of corn plants in the silking stage on 3 July. Eggs and 1st instars of H. zea and nymphs and adults of O. insidiosus were present on the silks at that time.

**Insect sampling.** At the control site, H. zea eggs and O. insidiosus females were collected from untreated sorghum interface plots on 8 and 11 July. At treated site 1, H. zea eggs were collected from sorghum interface plots on 8 and 11 July (20-23 d after RbCl application). No predators were collected at this field site. At treated site 2, H. zea eggs were collected from sorghum interface plots on 25 July and 1 August (22-29 d after RbCl application). Female O. insidiosus were collected from these interface plots at this site on 8 and 11 July (5-8 d after RbCl application). At this same site, H. zea eggs also were collected from cotton interface plots and cotton field plots on 25 July and 1 August. Females of O. insidiosus were collected from cotton field plots on 15 and 18 July (12-15 d after RbCl application) at this site. Unfortunately, O. insidiosus females were inadvertently not sampled in cotton interface plots at this location.

To collect insects, sorghum panicles were cut and placed in paper bags that were stapled closed, transported to the laboratory, and stored at 15°C. In the laboratory, all insects were removed from a sorghum panicle within 24 h of field collection following the technique described by Tillman and Mullinix (2004). Adult females of O. insidiosus and heliothine eggs (1-2 d old) were collected. Corn earworm eggs were identified to species following Neunzig’s (1969) description of eggs for this heliothine species. In cotton, whole plant sampling was used to sample insects. All H. zea eggs and O. insidiosus females found on cotton plants during the sampling procedure were collected. All collected insects of both species were placed separately in 1.5-mm centrifuge micro tubes (Fisher Scientific, Suwanee, GA) and stored in a freezer until analysis (up to 6 months at approximately −16°C). Voucher specimens of all insects are held in the USDA-ARS, Crop Protection & Management Research Laboratory in Tifton, GA.

**Sample preparation and analysis of rubidium content.** Orius insidiosus females and H. zea eggs were digested and analyzed for total rubidium content via atomic absorption spectrometry (AAS). All samples were dried in an oven maintained at ~45°C for 72 h. Orius insidiosus and some of the H. zea control samples were then massed to 1 µg precision. Because the mean mass for H. zea eggs was 0.017 µg with a standard deviation of only 0.003, the average was used on the samples that were not massed individually. After drying, 60 and 30 µL of concentrated HNO₃ (69%) were added to each O. insidiosus and H. zea sample, respectively. Samples were then returned to the oven maintained at 30-35°C for 24 h. Then 90 and 45 µL of H₂O₂ (30%) was added to each O. insidiosus and H. zea sample, respectively, to complete sample digestion. Samples were again placed in the oven for 24 h. After digestion, most samples were about 60 µL in total volume for O. insidiosus and 35 µL in total volume for H. zea. Enough reverse osmosis filtered water was added to produce samples of 100 µL total volume for O. insidiosus and 50 µL total volume for H. zea.

After sample preparation, Rb content was measured in a graphite furnace by AAS.
using a SpectrAA Zeeman 220 spectrometer (Varian Instruments, Sugar Land, TX) and following the technique described by Prasifka et al. (2001). Samples were analyzed for Rb content using subsamples of 10 μL repeated up to 3 times. This gave a Rb content in μg Rb/Liter of solution (digested sample). This value was converted to μg Rb/g of *O. insidiosus* using the masses that were obtained earlier for each sample; the spectrometer reading was multiplied by the sample volume (0.1 ml for the predator and 0.05 for the pest) and divided by the sample dry mass in mg. For the control *O. insidiosus* and *H. zea*, the μg Rb/g readings were used to generate a mean and SD for background Rb content. For the pest, there were some aberrant high values for the control samples, and these were omitted from data analysis because they were over 3 SD outside the mean. For *H. zea* eggs, two groups of samples were analyzed in the laboratory. The second group of samples had lower overall RbCl levels. The simplest explanation was that there was some contamination in the agents used to digest the first batch of samples. To compensate for this, the level of contamination was estimated by comparing the means of two field collected batches of control eggs. The difference was subtracted from samples run in the first group with values higher than the mark threshold to more accurately assess mark status. For both species, an insect was considered marked if its rubidium level exceeded the mean background plus three standard deviations, as made customary by Stimmann (1974).

Results and Discussion

Effectiveness of rubidium marking. Both *H. zea* eggs and *O. insidiosus* females were successfully marked in Rb-treated corn. For *H. zea* eggs at the control site, the mean rubidium content was 3.874 μg Rb/g of *H. zea* with a SD of 2.17. Thus, any *H. zea* egg with a rubidium content higher than 10.384 μg Rb/g (mean + 3 SD) was considered to be a marked sample (Table 1). Marking success for eggs of the pest ranged from 15.38-33.33%. In an earlier field study, Graham et al. (1978b) demonstrated that *H. zea* larvae and moths could be labeled with rubidium by treating corn with foliar applications of RbCl. Our study indicates that elevated levels of rubidium crossed through two generations from larva that fed upon treated corn to eggs from the females of the larvae that fed on Rb-treated corn.

### Table 1. Rubidium content of *H. zea* eggs in sorghum interface plots (SOR-I), cotton interface plots (COT-I), and cotton field plots (COT-F)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site*</th>
<th>Dates collected</th>
<th>No. eggs collected</th>
<th>Mean PPB Rb ± SD</th>
<th>Mark threshold</th>
<th>No. eggs marked</th>
<th>% eggs marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>7/8, 7/11</td>
<td>54</td>
<td>3.874 ± 2.170</td>
<td>10.384</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOR-I</td>
<td>1</td>
<td>7/8, 7/11</td>
<td>60</td>
<td>12.439 ± 11.823</td>
<td>20</td>
<td>33.33</td>
<td></td>
</tr>
<tr>
<td>SOR-I</td>
<td>2</td>
<td>7/25, 8/1</td>
<td>26</td>
<td>8.539 ± 7.197</td>
<td>4</td>
<td>15.38</td>
<td></td>
</tr>
<tr>
<td>COT-I</td>
<td>2</td>
<td>7/25, 8/1</td>
<td>24</td>
<td>6.670 ± 5.059</td>
<td>8</td>
<td>32.00</td>
<td></td>
</tr>
<tr>
<td>COT-F</td>
<td>2</td>
<td>7/25, 8/1</td>
<td>29</td>
<td>3.632 ± 1.697</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* At site 3, control *H. zea* eggs were collected from untreated sorghum interface plots. At site 1, RbCl solution was applied on 18 June 2002 on corn ears with mid to late-instar *H. zea*. At site 2, RbCl solution was applied on 3 July 2002 on corn ears with *H. zea* eggs on silks.
In our study, the percentage of marked eggs in sorghum was numerically higher when *H. zea* larvae were present in corn at application of RbCl than when *H. zea* eggs infested treated corn. Therefore, the success of marking may be greater when RbCl is applied to corn infested with larvae than with eggs. Graham et al. (1978b) reported that three weekly applications of 5.0 kg of RbCl/ha to corn plants beginning when the corn ears were infested with eggs were superior to a single application of 10 kg/ha made when corn ears were infested with larvae for labeling *H. zea* with rubidium indicating that the success of marking may be greater with multiple applications versus a single application. Nevertheless, as in the present study, corn was treated some time during the period when *H. zea* larvae were feeding on the ears regardless of the number of applications applied to corn.

At the control site, the mean (± SD) rubidium content for *O. insidiosus* females was 0.0908 (± 0.29) µg Rb/g of *O. insidiosus*. Any *O. insidiosus* female with a rubidium content higher than 1.778 µg Rb/g (mean ± 3 SD) was considered a marked sample (Table 2). Marking success for females of the predator ranged from 15.63-33.08%. Timing application of RbCI when the predator and its prey were abundant in corn silks probably increased the likelihood of successfully marking the predator. Previous field studies have demonstrated that *Orius* spp. can be successfully marked with rubidium in sorghum and cotton (Graham et al. 1978a, Prasifka et al. 2001), and our study demonstrated they can also be marked in corn.

**Insect dispersal.** At field site 1, RbCI was applied to ears of corn when mid to late *H. zea* instars were actively feeding on these fruiting structures. Eggs of *H. zea* were first observed on sorghum panicles in sorghum interface plots adjacent to corn 17 d later on 5 July. Soon after (3-6 d) these eggs first appeared in sorghum plots, 60 *H. zea* eggs were collected from sorghum panicles, and 33.33% of these eggs were internally marked with rubidium (Table 1). These data indicate that *H. zea* females from the generation feeding on rubidium-treated corn dispersed into sorghum interface plots and oviposited on sorghum panicles at this site.

At the second field site, RbCI was applied to ears of silking corn with *H. zea* eggs and 1st instars and *O. insidiosus* nymphs and adults on the silks. Eggs of *H. zea* eggs were observed for the first time on sorghum panicles in sorghum and cotton interface plots 19 d after application of RbCI, on 22 July. Eggs of this pest were collected from sorghum interface plots, cotton interface plots, and cotton field plots 3 and 7 d later. Of the eggs from sorghum, 15.38% were marked with rubidium whereas 32% of those from the cotton interface plots were marked (Table 1). None of the eggs collected

**Table 2. Rubidium content of *O. insidiosus* females in sorghum interface plots (SOR-I) and cotton field plots (COT-F)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site*</th>
<th>Dates females collected</th>
<th>No. females marked</th>
<th>Mean Rb ± SD</th>
<th>Mark threshold</th>
<th>% Females marked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>7/8, 7/11</td>
<td>40</td>
<td>0.908 ± 0.290</td>
<td>1.778</td>
<td></td>
</tr>
<tr>
<td>SOR-I</td>
<td>2</td>
<td>7/8, 7/11</td>
<td>130</td>
<td>4.905 ± 16.073</td>
<td>43</td>
<td>33.08</td>
</tr>
<tr>
<td>COT-F</td>
<td>2</td>
<td>7/15, 7/18</td>
<td>64</td>
<td>1.809 ± 4.450</td>
<td>10</td>
<td>15.63</td>
</tr>
</tbody>
</table>

* At site 3, control *O. insidiosus* females were collected from untreated sorghum interface plots. At site 2, RbCl solution was applied on 3 July 2002 on silking corn ears with *O. insidiosus* nymphs and adults.
from cotton field plots were marked with rubidium. These data indicate that *H. zea* females from the generation feeding on rubidium-treated corn dispersed into sorghum interface plots and oviposited on sorghum panicles at the second site also. In addition, female *H. zea* dispersed into cotton interface plots and oviposited on cotton plants. Occurrence of marked eggs in cotton interface plots and absence of them in cotton field plots may have been due to the fact that cotton in the field plots was further away from the rows where rubidium-marked females were emerging than cotton next to these treated corn rows. Perhaps the sample size was too low to detect the presence of marked eggs in the large cotton fields.

Five days after RbCl application at site 2, *O. insidiosus* females were observed on and then collected from sorghum panicles in the interface plots. The percentage of females from these plots which were marked by rubidium was 33.03% (Table 2). Females of this predator were found on and collected from cotton field plots 12 d later. Of the 64 *O. insidiosus* females captured in these plots, 15.63% of them were marked with rubidium (Table 2). These results indicate that this predator dispersed from maturing corn into the newly-available vegetation, sorghum panicles and cotton plants. At each of the 3 field sites, *O. insidiosus* were observed preying on eggs of this pest on corn silks in corn fields adjacent to the sorghum plots. Thus, rubidium-marked *O. insidiosus* adults in sorghum and cotton probably had previously ingested RbCl through sucking on rubidium-treated *H. zea* eggs or larvae or on rubidium-treated plant tissues in corn.

Our results demonstrate that rubidium marking can be used to detect dispersal of *H. zea* and *O. insidiosus*. In an earlier study on the dispersal of *H. zea* adults from a corn field labeled with rubidium, Rb-marked males were collected in traps baited with virgin females in cotton fields 0.5-2.8 km from the treated corn (Graham et al. 1978b). So apparently, *H. zea* moths from the larval generation that fed on Rb-treated corn dispersed from the corn field into cotton fields. Because Rb-marked *H. zea* eggs were found in sorghum and cotton adjacent to Rb-treated corn fields in our study, it is highly likely that female moths dispersed from corn into sorghum and cotton plots and also oviposited on sorghum panicles and cotton plants. These data indicate that corn can be a source of *H. zea* infestations in sorghum and cotton. Rubidium also has been used to detect dispersal of other pest species including the potato tuberworm in potato (Coll et al. 1997) and the boll weevil, *Anthonomus grandis* Boheman, in and around a cotton field (Wolfenbarger et al. 1982). In addition, this method was used to detect movement of natural enemies such as the aphid parasitoid, *Lysiphlebus testaceipes* (Cresson), in sorghum (Fernandes et al. 1997) and 8 different predator taxa, including *O. insidiosus*, between cotton and sorghum field plots (Prasifka et al. 2001). Our study provides further evidence that rubidium marking is a powerful and versatile technique for detecting the dispersal of insects.

Acknowledgments

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