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# Differential response of male and female *Diabrotica speciosa* (Coleoptera: Chrysomelidae) to bitter cucurbit-based toxic baits in relation to the treated area size

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Cucurbitacin-rich extracts are phagostimulants for both male and female Luperini, but field trials reveal that males are far more attracted to them than females. The sex ratio and number of beetles killed by an application of cucurbitacin-based toxic baits was assessed at two different scales: small areas of 100 $m^2$, and a larger area of 10,000 $m^2$. The small area bait applications killed mostly males (95.2%), but the 1-ha treated plot showed a lower sexual bias (57% male). An earlier study indicated point sources (sticky traps) attracted only males. Results indicate that the distance attraction to bitter cucurbit extracts is almost exclusive to males, but the wide dispersal of a toxic bait based on them should promote encounter and control of both sexes within the treated area. No significant non-target effects were observed.

**Keywords:** behavioural dimorphism; cucurbitacin bait; kairomones; non-target effects; phagostimulants

## 1. Introduction

The tribe of leaf beetles, Luperini (Coleoptera: Chrysomelidae: Galerucinae), contains several pest species, notably the subtribe Diabroticina. Regardless of the hosts and geographical distribution of these beetles, most of them have a compulsive feeding response to cucurbitacins, which are toxic triterpenoids that function as plant chemical defences, primarily in the family Cucurbitaceae (Contardi 1939; Howe et al. 1976; Metcalf and Lampman 1989). Several pest management applications have been implemented based on this behavioural response. These include lacing cucurbit roots or fruit with an insecticide (Lorenzato 1984; Hamerschmidt 1985), using them in traps for monitoring and collecting Luperini (Shaw et al. 1984; Cabrera Walsh and Cabrera 2004; Cabrera Walsh 2012), and bitter cucurbit juice formulations combined with insecticides (Metcalf et al. 1987; Lance and Sutter 1990; Barbercheck et al. 1995; Schroder et al. 2001; Pedersen and Godfrey 2011). Toxic baits containing cucurbitacin-rich juices or powders were commercially available until a few years ago under at least five brand names and several formulations. The cucurbitacin-based toxic baits could reduce the use of insecticides per hectare to 2−5% of the recommended rates for adult *Diabrotica* suppression (McKenzie et al. 2002). Another positive feature of these baits is that they have shown no negative impact on non-target arthropods (McKenzie et al. 2002, Boetel et al. 2005), making them compatible with integrated pest management programmes (Lewis et al. 2005).

The results obtained with the application of these baits to control *Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence were initially very auspicious. However, differences between the levels of larval damage to the roots the following season in treated and untreated maize were sometimes non-significant (Chandler 2003; Gerber et al. 2005). Parimi et al. (2003) proposed that a history of previous resistance to carbaryl, used as the toxicant in the bait, could have compromised its efficacy. Behle (2001) reported that twice as many males than females were killed by a cucurbitacin + insecticide bait. This difference was attributed to females being less susceptible to the insecticide, possibly owing to a larger body size. Finally, Gerber et al. (2005) stated that the residual activity of the bait was probably insufficient to guarantee sufficient levels of adult control to reduce larval damage from the ensuing generation, indicating that this aspect of the formulations needed improvement.

The effectiveness of these baits, however, may depend critically on the differential attraction of male and female *Diabrotica*. Several studies report that the adults of three species of North American *Diabrotica* found on cucurbitacin sources were mostly males (Branson and Guss 1983; Fielding and Ruesink 1985; Shaw et al. 1984; Tallamy and Halaweish 1993; Tallamy et al. 2000, 2002). This male preponderance in the catches has usually been attributed to males being more active (Branson and Guss 1983; Fielding and Ruesink 1985; Tallamy and Halaweish 1993), or to mating status related to the fact that Dibroticine males are known to pass cucurbitacins to females during mating (Tallamy and Halaweish 1993).

For the South American species *Diabrotica speciosa* (Germar) and *Diabrotica viridula* (F.), choice tests

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showed that males of these two species would ingest cucurbitacins in greater amounts and for longer periods of time than females (Cabrera Walsh and Cabrera 2004). Also, there is in the field a marked male dominance on cucurbit plants, and an even higher male proportion on traps baited with different bitter cucurbit preparations of both South and North American species (Cabrera Walsh 2003; Cabrera Walsh and Cabrera 2004; Cabrera Walsh et al. 2008). The evidence indicates that there is a distinct sexual difference in the response of Luperini to cucurbitacin sources. Point sources of the cucurbitacin rich bait in the form of a sticky trap attracted males from a distance of up to 30 metres, but virtually no females of *D. speciosa* were caught (Cabrera Walsh et al. 2014).

Most publications analysing the results of cucurbitacin bait applications have not addressed this factor (Zhu et al. 2001; Chandler 2003; Siegfried et al. 2004; Arruda et al. 2005; van Rozen and Ester 2010). Yet, limited attraction of *Diabrotica* females to cucurbitacins could jeopardize the success of baits, depending on their deployment. Adult survival and density normally defines the density of the next generation. However, according to calculations by Sutter and Lance (1991), 44% female *D. v. virgifera* survival is enough to ensure similar population levels for the next generation. Therefore, this response difference could decrease the efficacy of cucurbitacin baits in reducing the field egg bank, even if a significant proportion of the adult population were eliminated.

In this work the expression of the male predominance of *D. speciosa* on cucurbit-based baits was tested at two different scales in order to evaluate the significance of the attraction disparity on the efficacy of said baits in the field. We compared sex ratios for 100-m$^2$ plots and a 1-ha plot treated with bitter cucurbit-based baits, and also assessed the non-target effects. The effects on other species of Diabroticina were also assessed when possible.

## 2. Experimental methods

### 2.1. *Test plots*

Four series of field experiments were performed with insecticide plus bitter cucurbit extract baits: three series of experiments with 100-m$^2$ treated areas were performed in a 5-ha alfalfa field and a 450-m$^2$ maize plot in Mercedes, Buenos Aires province (34°34′19″S; 59°21′17″W). These crops had never been treated with insecticides.

A fourth experiment covering a 1-ha area was set in a 44-ha butternut squash field north of the city of Orán, Salta province (22°55′10″S; 64°22′09″W). This field had not been sprayed for 15 days before the establishment of the experiment.

During the experiments in Mercedes, a sample of Diabroticina was collected on every inspection date in the study areas using a standard sweep net (30-cm opening) and taking 500 sweeps. These samples were counted and sexed in order to obtain a measure of the relative beetle density and the expected sex ratio in the crop on any given date. Each sample was taken at least 50 m away from the treated areas. In Orán, 500 Diabroticina were collected with aspirators from squash flowers 50+ metres away from the treated plots. Randomness of the sample was ensured by aspirating indiscriminately all the insects from the flowers. This sample was preserved in 70% EtOH, and sexed and identified to species level in the laboratory.

### 2.2. *Bait applications*

The bait was formulated as in Schroder et al. (2001), with 100 g of carbaryl (Sevin 85 S®, Bayer CropScience, Leverkusen, Germany), 1 g of gelva multipolymer emulsion (Monsanto, St. Louis, Missouri), and 3 g of modified starch (Mira Sperse 626®, A.E. Staley Mfg. Co., Decatur, Illinois) in 10 litres of liquid (cucurbit extracts described below; or water in the control treatments) per hectare. The emulsion was applied in coarse droplets with manual backpack sprayers.

In the Mercedes plots (small plots), 100 m$^2$ of maize was sprayed on 1 April and 5 April (trials A and C respectively); and 100 m$^2$ of alfalfa on 4 April 2006 (trial B), with a bait containing *Cucumis myriocarpus* Naudin juice, extracted from fruit grown in our laboratory greenhouses in Hurlingham, Argentina (for details on the extraction process see Cabrera Walsh et al. 2008). Before applying the bait, several polyethylene strips brushed with an adhesive emulsion (paraffin 2%, linseed oil 44%, rosin 54%) were spread between the treated maize rows, and along mowed strips within the treated alfalfa plots. Each plot contained 16 × 0.5 m$^2$ of sticky strips. These strips prevented scavengers from taking the cadavers, and the rain and wind from sweeping them away, thus allowing the recovery of a significant sample of target beetles and all other insects found within the bait and control treatments. The arthropods collected from the sticky strips were rinsed in edible oil, and preserved in 70% EtOH for identification in the laboratory. Dead beetles observed on treated leaves were also collected.

In the Orán plot (large plots), 1 ha of mature squash was divided into 9 equal square plots. These plots (each approximately 1111 m$^2$) were assigned to three treatments at random: three to a toxic bait formulated with *C. myriocarpus* fruit juice, three to a toxic bait formulated with *Cayaponia bonariensis* (Miller) Martínez Crovetto root juice, and three controls (details described in the next section). Each plot had a single polyethylene sticky strip measuring 8 × 0.5 m$^2$ placed at random to trap Luperini adults and non-target arthropods. Arthropod cadavers found on the strips and the treated squash plants were collected and preserved in 70% EtOH for identification in the laboratory.

### 2.3. *Controls*

The controls used at the Mercedes plots consisted of the same length of sticky strips as in the treated plots, spread at random between rows of untreated maize and alfalfa. By utilizing these strips, it was possible to assess the number of arthropods accidentally killed by the sticky strips alone, subtracting the number of arthropods on the sticky strips in the untreated plots from those obtained in the treated sectors. These plots were located adjacent to the bait treated plots, with a buffer zone of 2 m.

The control treatment used in the large area bait tests in Orán consisted of an emulsion formulated as in the treated plots, but with water instead of bitter cucurbit extract. This control treatment was used to evaluate arthropods killed by the insecticide in the absence of any cucurbitacin feeding stimulant. As in the plots treated with baits, each control plot had one 8 × 0.5 $m^2$ sticky strip.

The number and sex of the dead adult Diabroticina collected on the leaves and sticky strips were recorded at 1, 12, 24 and 36 hours after application. The non-target arthropods caught on the sticky strips from the treated and control plots were counted and identified to family level, except for millipedes and spiders, which were only identified as Diplopoda and Araneae, respectively.

### 2.4. *Statistical analyses*

The numbers of *D. speciosa* of both sexes killed at the Orán plot by the *C. myriocarpus* and *C. bonariensis* baits were tested for normality and compared by analysis of variance (ANOVA) using sampling time and extract type as factors, followed by Bonferroni-corrected multiple comparisons (Systat 2004). The numbers of *Diabrotica speciosa* killed in the control plots, as well as the other Diabroticina species found in the plots, were not included in these analyses because of the preponderance of zeros per plot.

The proportion of *D. speciosa* of each sex killed by the bait in each of the three Mercedes (100 $m^2$) trials was modelled using logistic regression to test for significant change in the sex ratio over time since treatment. At each of the five sample times, each collection (number of male and female *D. speciosa*) was compared by Fisher's exact test to the overall number of males and females captured by sweep net sampling during the 120-hour sampling period.

The proportion of *D. speciosa* of each sex killed by the bait at the Orán plot (1 ha) was modelled for each treatment (*Cucumis*-based and *Cayaponia*-based bait) using logistic regression (SAS Institute 1998) to test for significant change in the sex ratio over time since treatment. In addition, at each of the four sample times, the two treatments were compared to the total male and female adults collected at the beginning of the sample period, using a 3 × 2 $\chi^2$ test, and if this was significant at the $\alpha = 0.0167$ level, multiple comparisons were carried out between aspirated sample totals and each treatment collection by Bonferroni-corrected Fisher's exact test ($\alpha = 0.0167 = 0.05/3$) (SAS Institute 1998).

The non-target arthropods found stuck to the sticky strips of the bait and control plots were compared with non-parametric tests, handling each treatment as a factor, and each family, plus Diplopoda and Araneae, as a case. The Orán bait applications were compared with a Kruskall–Wallis test, and the applications in the Mercedes plots were compared with Mann–Whitney *U* tests. The non-targets found dead on foliage in the Orán experiment were pooled per treatment and compared with a Kruskall–Wallis test, considering sampling periods as cases (Systat 2004).

## 3. Results

### 3.1. *Controls*

The controls yielded zero *D. speciosa* adults in the Mercedes applications, against 393 in the treated plots; and controls showed only 14 dead adults in the Orán application, against 580 Luperini of different species in the treated plots; all of them on the adhesive strips. In view of these results, no statistical analyses were employed for this aspect of the study, and all the dead Diabroticina observed in the treated plots were considered victims of the toxic bait.

### 3.2. *Mercedes small-scale application*

*Effect of the bait on males and females.* In the Mercedes experiments, the presumed source population sampled by sweep-netting was 60.5% male ($n = 223$). In all three trials at all five collection times, males were still more abundant than this sweep sample mean, with a mean proportion of 95.4% males ($n = 846$). Every collection for 24, 48 and 120 hours post-treatment exceeded 90%, and was significantly different from the sweep sample mean by Fisher's exact test at $P < 0.0001$ (Figure 1). For each of the maize trials (A and C), the proportion of males

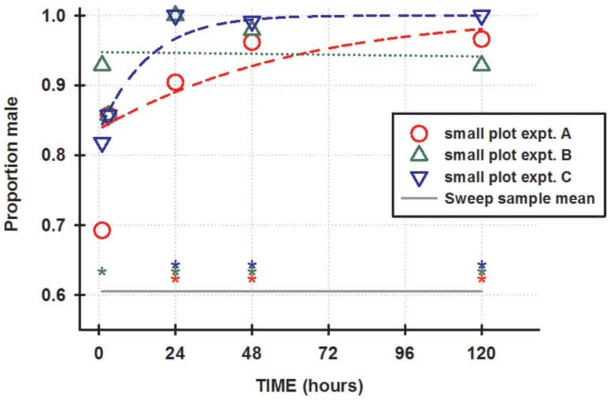

Figure 1. Proportion of males from of *Diabrotica speciosa* adults killed at 1, 3, 24, 48, and 120 h post-treatment by the cucurbitacin bait in the Mercedes bait application plots. Logistic regressions (coloured dashed lines) shown indicate that the proportion of males increased significantly over time from the 1-h through the 120-h post-treatment samples for each of the three experimental trials [Trial A: $\beta_0 = 1.638$, $\beta_1 = 0.019$, $\chi_1^2 = 6.33$, $P = 0.012$; Trial B: $\beta_0 = 2.893$, $\beta_1 = -0.001$, $\chi_1^2 = 0.077$, $P = 0.781$ (n.s.); Trial C, $\beta_0 = 1.617$, $\beta_1 = 0.073$, $\chi_1^2 = 16.48$, $P < 0.0001$, where $P(\text{male}) = 1/(1 + e^{-(\beta_0+\beta_1 x)})$]. Colored asterisks indicate those proportions which differed by Fisher's exact test (in all cases, $P < 0.0001$) from the proportion of males in the overall sweep sample mean of local populations.

increased significantly over time, according to the logistic regression. In the alfalfa trial, there was no significant time trend, although the overall proportion of male was 94.3%. (Figure 1 and caption).

*Effect of the bait on non-targets.* In the Mercedes plots, where the controls tested the number of accidental victims of the sticky strips, the number and groups of arthropods caught on the sticky strips in treated and untreated plots were not significantly different in the maize (Mann−Whitney $U = 231.5$, $P = 0.781$) or alfalfa plots (Mann−Whitney $U = 193$, $P = 0.849$) (Table 1).

### 3.3. Orán large-scale application

*Effect of the bait on males and females.* In the Orán trial, the presumed source population, aspirated at time zero, was 32.6% male ($n = 282$). In comparison, adults killed in both treatments exceeded this proportion of males at 24 and 48 hours, but at no sample time did the two bait treatments (*Cucumis* and *Cayaponia*) differ in proportion by Fisher's exact test at $\alpha = 0.0167$ (Figure 2). The proportion of males increased over time (Table 2, Figure 2). For both of the cucurbit bait treatments, logistic regression showed that the proportion of males increased over time from the 1 through the 36-hour post-treatment samples (*Cucumis* treatment, $\beta_0 = -0.555$, $\beta_1 = 0.029$, $\chi_1{}^2 = 7.96$, $P = 0.0048$; *Cayaponia* treatment, $\beta_0 = -0.053$, $\beta_1 = 0.034$, $\chi_1{}^2 = 10.18$, $P = 0.0014$; where $P$ (male) $= 1/(1 + e^{-(\beta_0+\beta_1 x)})$.

The two-way ANOVA of the number of total dead individuals of *D. speciosa* showed no significant effect of the extract type ($F = 3.13$, d.f. $= 1$, $P = 0.096$) or interaction between time and extract ($F = 1.351$, d.f. $= 3$, $P = 0.293$). The *Cucumis* juice bait and *Cayaponia* juice bait killed 260 and 236 beetles of *D. speciosa* after 36 hours, respectively (Table 3).

*Effect of the bait on other Diabroticina and non-targets.* Apart from *D. speciosa*, the bait also killed Luperini

Table 1. Non-target arthropods collected on sticky strips in the small-scale plots.

| Maize | | | Alfalfa | | |
|---|---|---|---|---|---|
| Non-target | Control | Treated | Non-target | Control | Treated |
| DIPTERA | | | DIPTERA | | |
| Calliphoridae | 5 | 8 | Phoridae | 546 | 625 |
| Muscidae | 9 | 11 | Calliphoridae | 2 | 2 |
| Tachinidae | 10 | 15 | Tachinidae | 2 | 1 |
| Tephritidae | 13 | 18 | Tipulidae | 3 | 2 |
| Tipulidae | 6 | 16 | LEPIDOPTERA | | |
| LEPIDOPTERA | | | Pyralidae | 8 | 3 |
| Gelechiidae | 3 | 2 | COLEOPTERA | | |
| Noctuidae | 9 | 1 | Coccinellidae | 1 | 4 |
| Pyralidae | 3 | 5 | Carabidae | 7 | 4 |
| COLEOPTERA | | | Chrysomelidae | 75 | 31 |
| Carabidae | 3 | 1 | Curculionidae | 12 | 9 |
| Coccinellidae | 0 | 2 | Elateridae | 1 | 2 |
| Curculionidae | 11 | 12 | Scarabaeidae | 0 | 2 |
| Ditiscidae | 3 | 0 | HEMIPTERA | | |
| Mordellidae | 3 | 0 | Aphididae | 390 | 412 |
| Nitidulidae | 6 | 18 | Cicadellidae | 12 | 27 |
| Scarabaeidae | 6 | 2 | Cercopidae | 1 | 2 |
| HEMIPTERA | | | HYMENOPTERA | | |
| Cicadellidae | 23 | 24 | Apidae | 3 | 2 |
| Reduviidae | 0 | 1 | Formicidae | 5 | 5 |
| HYMENOPTERA | | | ORTHOPTERA | | |
| Formicidae | 26 | 5 | Acrididae | 3 | 6 |
| ORTHOPTERA | | | Blattidae | 1 | 0 |
| Acrididae | 0 | 1 | NEUROPTERA | | |
| Gryllidae | 26 | 14 | Chrysopidae | 1 | 1 |
| DERMAPTERA | | | DIPLOPODA | 14 | 29 |
| Forficulidae | 26 | 61 | | | |
| NEUROPTERA | | | TOTAL | 1087 | 1169 |
| Chrysopidae | 0 | 2 | | | |
| DIPLOPODA | 75 | 85 | | | |
| ARANEAE | 46 | 24 | | | |
| TOTAL | 312 | 328 | | | |

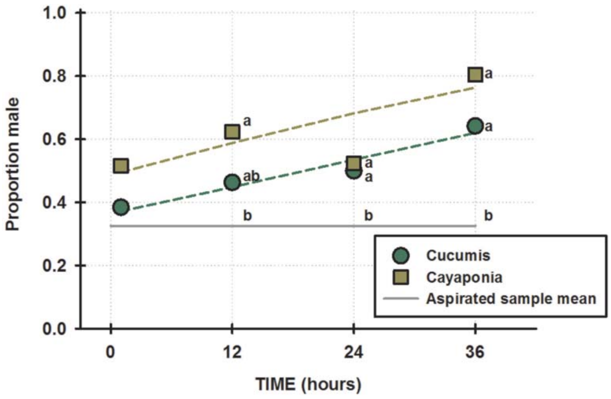

Figure 2. Proportion of males from *Diabrotica speciosa* adults killed by the two different cucurbitacin baits in large-scale plots, compared to the proportion in the field-aspirated sample at time 0. Logistic regressions (dotted) shown indicate that the proportion of males increased significantly over time from the 1-h through the 36-h post-treatment samples [*Cucumis* treatment: $\beta_0 = -0.555$, $\beta_1 = 0.029$, $\chi_1^2 = 7.96$, $P = 0.0048$; *Cayaponia* treatment: $\beta_0 = -0.053$, $\beta_1 = 0.034$, $\chi_1^2 = 10.18$, $P = 0.0014$; where $P(\text{male}) = 1/(1+e^{-(\beta_0+\beta_1 x)})$]. Letters indicate samples differing by Bonferroni-corrected Fisher's exact test ($\alpha = 0.0167 = 0.05/3$).

of the species *Paranapiacaba significata* (Gahan), *Acalymma bivittula* (Kirsch), *Acalymma bruchii* (Bowditch), *Cerotoma arcuata* Olivier, and *Buckibrotica* sp. (Table 3).

In the Orán plot, approximately the same groups, and roughly the same numbers, of arthropods were killed in the treated (insecticide + bait) and control (insecticide + water) plots (Table 4). A total of 65 non-target arthropods were found dead on the leaves in the Orán experiment, 45 in the 6 bait-treated plots, and 20 in the 3 control plots. In the 6 treated plots there were 3591 non-targets on the sticky strips, and 1657 in the 3 control plots. As with the Mercedes plots, no significant differences were found between the bait-treated plots and the control plots (Kruskall–Wallis $K = 4.095$, d.f. $= 2$, $P = 0.129$). In contrast, 526 Diabroticina adults were collected dead on leaves, with only 54 collected on the sticky strips.

Table 2. Summarized results of the 1-ha bait application plot in Orán, Salta province: number of *Diabrotica speciosa* adults found dead in the treated plots at each inspection.

| Time | Treatment | Males | Females |
|---|---|---|---|
| 1 h | *Cucumis* | 15 | 24 |
| | *Cayaponia* | 17 | 16 |
| | Control | 1 | 4 |
| 12 h | *Cucumis* | 25 | 29 |
| | *Cayaponia* | 43 | 26 |
| | Control | 2 | 1 |
| 24 h | *Cucumis* | 43 | 43 |
| | *Cayaponia* | 22 | 20 |
| | Control | 1 | 0 |
| 36 h | *Cucumis* | 52 | 29 |
| | *Cayaponia* | 74 | 18 |
| | Control | 3 | 2 |

Table 3. Summarized results of the 1-ha bait application plot in Orán, Salta province: total numbers of Diabroticina of each species killed in the treated plots.

| Treatment | Species killed | Males | Females |
|---|---|---|---|
| *Cucumis* | *D. speciosa* | 135 | 125 |
| | *Paranapiacaba* | 5 | 8 |
| | *Acalymma* | 6 | 9 |
| | *Cerotoma* | 11 | 7 |
| *Cayaponia* | *D. speciosa* | 156 | 80 |
| | *Paranapiacaba* | 3 | 2 |
| | *Acalymma* | 2 | 2 |
| | *Cerotoma* | 5 | 7 |
| | *Buckibrotica* | 1 | 1 |
| Control | *D. speciosa* | 7 | 7 |
| | *Paranapiacaba* | 0 | 0 |
| | *Acalymma* | 0 | 1 |
| | *Cerotoma* | 0 | 0 |

## 4. Discussion

Our results for both the Mercedes (small-scale) and Orán (large-scale) applications, show that at 24 hours onwards, after the application of cucurbitacin-containing toxic baits, in every case (16 of 16 samples), the male proportion of *D. speciosa* killed exceeded that of the background (presumed source) population. This difference is very clearly greater (comparing Figure 1 to Figure 2) in the Mercedes trials compared with the Orán plots. Small area bait applications killed a small proportion of females at the beginning of the test (11.6% proportion of total for 1 and 3 hours), and then very few (3.0% in total, and in every case less than 10% females) at 24 hours and afterwards, suggesting that the females in the treated plots encountered and fed on the bait, and died, depressing the number of females in the treated area. The number of dead males continued to increase in the two maize plots over time, as demonstrated by the positive logistic regressions, and remained high, though not significantly increasing, for the alfalfa plot. This pattern presumably reflects migration of additional males into the treated plots for several days.

In the Orán plot, proportions of dead males sampled increased significantly over time, and were significantly above background sampled proportions at the 24 h and 36 h sampling times (Figure 2). However, the proportion was not as different in bait-killed dead beetles as it was in Mercedes. In other words, the larger the treated area, the closer the sex ratio of beetles killed approached the ratio present in the field. This suggests that as long as the females encountered cucurbitacin, feeding occurred. More importantly, results also suggest that the females in the application zone will suffer similar mortality to that of males, with the male numbers higher from being attracted into the treated area. The extreme case is a single point source – a trap – in which all captures are based on attraction, not random encounter; in this case, the baits were completely ignored by the females (Cabrera Walsh et al. 2014).

Table 4. Non-target arthropods collected on sticky strips in the 1-ha plot in Orán.

| Non-target | Control | *Cayaponia* | *Cucumis* |
|---|---|---|---|
| DIPTERA | | | |
| Calliphoridae | 12 | 15 | 6 |
| Culicidae | 1 | 0 | 0 |
| Chironomidae | 23 | 5 | 3 |
| Drosophilidae | 8 | 1 | 0 |
| Muscidae | 18 | 9 | 11 |
| Phoridae | 1,320 | 1,372 | 1,632 |
| Pipunculidae | 3 | 0 | 2 |
| Stratyomyidae | 1 | 2 | 0 |
| Tachinidae | 22 | 12 | 8 |
| Tephritidae | 1 | 0 | 0 |
| Tipulidae | 19 | 17 | 7 |
| LEPIDOPTERA | | | |
| Crambidae | 19 | 17 | 15 |
| Noctuidae | 10 | 12 | 18 |
| Noctuidae (larvae) | 1 | 0 | 1 |
| HEMIPTERA | | | |
| Cicadellidae | 21 | 15 | 6 |
| Miridae | 10 | 3 | 0 |
| Cercopidae | 3 | 1 | 3 |
| Reduviidae | 1 | 0 | 1 |
| COLEOPTERA | | | |
| Carabidae | 11 | 5 | 8 |
| Chrysomelidae | 2 | 0 | 1 |
| Curculionidae | 1 | 2 | 1 |
| Dasytidae | 0 | 1 | 0 |
| Dytiscidae | 57 | 50 | 179 |
| Elateridae | 3 | 11 | 5 |
| Mordellidae | 23 | 16 | 14 |
| Nitidulidae | 2 | 14 | 0 |
| Scarabaeidae | 3 | 2 | 0 |
| Staphylinidae | 0 | 2 | 2 |
| HYMENOPTERA | | | |
| Formicidae | 6 | 2 | 1 |
| Ichneumonoidea | 2 | 0 | 1 |
| Vespidae | 0 | 2 | 0 |
| ORTHOPTERA | | | |
| Blattidae | 1 | 1 | 0 |
| Gryllidae | 34 | 27 | 30 |
| Tettigonidae | 4 | 2 | 2 |
| DERMAPTERA | | | |
| Forficulidae | 7 | 6 | 9 |
| ODONATA | | | |
| Coenagrionidae | 1 | 0 | 0 |
| DIPLOPODA | 1 | 0 | 1 |
| ARANEAE | 6 | 2 | 0 |
| TOTAL | 1657 | 1624 | 1967 |

Another interesting result of these experiments is that Luperini species that are not usually attracted to point sources of cucurbitacin (Cabrera Walsh et al. 2008) will succumb to the “inundative” effect of the bait application. For both sexes of *Acalymma* spp., *P. significata* and *C. arcuata*, the cucurbitacin-containing bait produced more mortality than the control containing only insecticide and water (Table 3). This suggests that widely-dispersed cucurbitacin baits will affect Diabroticina of low sensitivity for cucurbitacins, as with *D. speciosa* females.

Non-target effects proved negligible in both experiments: the sticky strips in untreated plots and the plots treated with 1% carbaryl in water. This indicates that the non-target arthropods ended up on the sticky strips in the treated plots accidentally, not the result of being attracted to the bait. The low number of non-targets found dead on leaves, as compared to the number of non-targets on the sticky strips, also suggests that the low concentration of carbaryl used in the bait did not adversely affect a significant number of arthropods. A slightly larger number of non-targets in one of the *Cucumis* plots was due to an unexpected number of Dytiscidae collected off one of the sticky strips (Table 4). We hypothesize that these water beetles confused the sheen of the sticky substance on the strips with water, and by landing on the strips were trapped accidentally. This particular strip had a higher sheen than the other strips because additional adhesive was applied to the strip after the wind overturned it, covering the entire strip with soil.

Previous publications indicate that females consume smaller quantities of cucurbitacin-enriched diet than males (Tallamy and Halaweish 1993; Cabrera Walsh and Cabrera 2004). However, Tallamy et al. (1997) reported that the gustatory sensitivity to cucurbitacin B was generally identical between the sexes of different North American Diabroticite species. In fact the few variations in sensitivity were more related to geographical origin than to gender. These experiments, performed in a 1.5 × 1 $cm^2$ arena, demonstrated that beetles of both sexes not previously exposed to cucurbitacins avidly fed on pure cucurbitacins for the duration of the experiment (10 min). Much of our knowledge regarding the relationship between Diabroticina and cucurbitacins is based on brief experiments conducted with pure cucurbitacins in confined situations (Metcalf et al. 1982; Nishida et al. 1986; Tallamy et al. 1997). But these laboratory experiments will not necessarily predict results in field situations, with the unrefined compounds used in experimental and commercial baits, and unconfined insects. Given the multiplicity of compounds found in unrefined bitter cucurbit extracts (Cossé and Baker 1999), and the physical and behavioural variables involved, there are likely to be significant differences in the stimuli received by the beetles in the field.

This study, combined with the preceding study using sticky traps (Cabrera Walsh et al. 2014), shows that cucurbitacin baited traps may be useful as monitoring tools and perhaps for the control of Diabroticina in situations where the adults are the problematic stage, as in horticultural crops, or to prevent yield loss from adult beetle silk clipping in maize. However, when the larvae are the main target, only methods that will kill a significant proportion of females could be of any use. Our work suggests that widely applied toxic baits would be the only suitable way of using cucurbitacins to obtain this result. However, current formulations probably need improvements in the insecticide choice, concentration, and/or residuality. In any case, in view of the growing complexities for controlling corn rootworms and cucumber beetles, and the demand for alternatives to insecticide applications, there seem to be several good reasons, both economic and environmental, for perfecting these baits and their use.

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