Effectiveness of GF-120NF Fruit Fly Bait as a suppression tool for *Bactrocera latifrons* (Diptera: Tephritidae)

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Abstract

*Bactrocera latifrons* (Hendel) is a tephritid fruit fly of primarily Asian distribution that has invaded Hawaii and, more recently, the continent of Africa (Tanzania and Kenya). It primarily infests solanaceous fruits, so has the potential to impact production of crops such as peppers (*Capsicum annuum* L. and *Capsicum frutescens* L.), eggplant (*Solanum melongena* L.), African eggplant (*Solanum aethiopicum* L.) and tomatoes (*Solanum lycopersicum* L.). Because little work has been done to develop suppression techniques for this fruit fly species, field cage tests of the effectiveness of a commercially available bait spray, GF-120NF Fruit Fly Bait, against wild *B. latifrons* were conducted. Sexually mature *B. latifrons* adults (75 male and 75 female) were introduced to both a control cage and a treatment cage, each of which held six fruiting Anaheim chili pepper (*C. annuum* L.) plants. Fruits were harvested, and assessed for infestation, both before and after the application of the bait spray in the treatment cage. There was no difference in infestation rate between control and treatment cages before the application of the bait spray, whereas there was a significantly lower infestation rate in treatment cages following the application of the bait spray. Post-spray infestation rate in the treatment cages (in two separate, replicated bioassays) was always zero and no live flies were detected in the treatment cages at the end of the trials. The results of this study provide evidence that GF-120NF Fruit Fly Bait should be effective in suppressing *B. latifrons* populations in the field.

Introduction

*Bactrocera latifrons* (Hendel) is a tephritid fruit fly that primarily infests solanaceous fruits but has also been found to infest some cucurbitaceous fruits (Liquido et al. 1994; Shimizu et al. 2007). It is of primarily Asian distribution (e.g. Pakistan, India, Sri Lanka, Burma, China, Thailand, Laos, Vietnam, Malaysia, Singapore, Taiwan and Brunei) (Carroll et al. 2002), but has invaded Hawaii, where it was first detected in 1983 (Vargas and Nishida 1985). Additionally, it has recently invaded the continent of Africa, where it was detected in Tanzania in 2006 (Mwatawala et al. 2007) and in Kenya in 2007 (De Meyer et al. 2007; S. Ekesi, personal communication). Although, at present, little economic damage has been attributed to this species in Hawaii, it is considered a quarantine pest resulting in the requirement for costly quarantine treatments for any host fruits shipped from Hawaii. It has the potential to impact production of solanaceous crops such as peppers (*Capsicum annuum* L. and *Capsicum frutescens* L.), eggplant (*Solanum melongena* L.) and tomatoes (*Solanum lycopersicum* L.), crops identified for diversified agriculture in Hawaii.

As crop production situations arise where population suppression of *B. latifrons* is needed, it is important to identify reliable suppression techniques. As
with other tephritid fruit fly species, it is expected that proteinaceous bait sprays would have some effectiveness in controlling *B. latifrons*. However, no trials using bait sprays have yet been conducted (McQuate et al. 2007). One environmentally friendly bait that is commercially available is the spinosad-based bait, GF-120NF Fruit Fly Bait (DowAgroSciences, Indianapolis, IN). Preliminary laboratory toxicology tests with laboratory colony flies have shown that mortality response of *B. latifrons* to GF-120NF Fruit Fly Bait is comparable to that of *Bactrocera cucurbitae* and *Bactrocera dorsalis* (McQuate, unpublished data). Earlier research has shown GF-120 Fruit Fly Bait to have some level of effectiveness against these other two tephritid fruit fly species (Prokopy et al. 2003; Barry et al. 2006). Additionally, field attraction trials have shown that GF-120 Fruit Fly Bait is attractive to adult *B. latifrons* (McQuate, unpublished data). Because these preliminary tests suggested that there was good likelihood that GF-120 Fruit Fly Bait would be effective in controlling *B. latifrons*, we sought to conduct confirmatory tests with wild flies, the results of which are presented here. Because we were unable to locate sites in Hawaii where we could conduct the trials in the field, our tests were conducted in field cages using wild flies.

**Materials and Methods**

**Chemicals**

GF-120NF Fruit Fly Bait (Dow AgroSciences, IN) was obtained locally. Additionally, a GF-120 Fruit Fly Bait blank which contained all ingredients except the toxicant (spinosad) was obtained directly from Dow AgroSciences.

**Insects**

All *B. latifrons* adult flies used in this study were recovered from fruits of wild turkeyberry, *Solanum torvum* (Sw.), collected from the vicinity of Haiku on the island of Maui, Hawaii. Fruits were transported to the laboratory at the Pacific Basin Agricultural Research Center on the island of Hawaii and subsequently held in screened buckets with sand on the bottom to serve as a pupation medium. Sand was sieved weekly for recovery of pupariating larvae and pupae which were then placed in screened-top cups and held in the laboratory at an average (± SEM) temperature of 23.3 (±0.02)°C and relative humidity (± SEM) of 54.3 (±0.11), and a photoperiod of 12 : 12 (L : D) hours. Flies emerging on successive days were removed and grouped in 0.30 m cubical screened-top cages to total at least 150 ♀ and 150 ♂. Adults were fed water, sucrose and a ‘protein cake’ [consisting of one part protein yeast hydrolysate (Enzymatic, United States Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhinelander Paper Co., Rhinelander, WI)] until they reached sexual maturity and were used in the field cage trials. Estimation of age of sexual maturity was based on results presented in McQuate et al. (2008).

**Field cage bioassays**

Two 1.8 × 1.8 × 1.8 m screened cages (BioQuip Products, Rancho Dominguez, CA), were placed 0.5 m apart underneath a 6.1 × 6.1 m silver tarp canopy suspended 2.5 m above the ground at the edges and 3.5 m above the ground in the middle. Anaheim chili pepper (*C. annuum* L.) plants were raised to maturity from locally purchased seedlings. Two bioassays were conducted, with each bioassay replicated two times. Each replication of each field cage bioassay was conducted over a 9-day period. The steps in the bioassays common to both bioassays are described below: On Day 0 of the bioassay, 12 plants with mature green fruits were selected and half of the fruits (longer than 2.0 cm) on each plant were individually bagged with two pound brown paper bags. Six of these plants were placed in each screened cage. On Day 1, 75 male and 75 female sexually mature adult *B. latifrons* flies were placed in each cage between 8 : 30 and 12 : 00 hours. On the morning of Day 3, all unbagged fruits from each cage were removed to provide a pre-spray assessment of ovipositional ability and fly fertility. Collected fruits were weighed individually and then placed in 4.0 l screen-topped buckets with sand on the bottom to serve as a pupariation medium, with all fruits from a given plant held together in the same holding container. After 3 weeks, fruits were processed for assessment of fruit fly infestation. Recovered pupae and pupariating larvae were transferred to 250 ml screen-topped containers and held until all adult emergence was completed, after which all emerged adults were sexed and counted. After removing the unbagged fruits, bait spray was applied to the underside of the leaves of each plant in each cage. In the treatment cage, GF-120NF Fruit Fly Bait was applied, whereas the GF-120 Fruit Fly Bait blank was applied in the control cage. In both cases, the bait was diluted four parts of bait to six parts of water. This is
the standard dilution recommended on the product label and produces a spinosad concentration of 89 ppm. On Day 5, the paper bags were removed from the remaining peppers on the plants in the cages to allow any surviving female flies to oviposit. On Day 7, all of the remaining peppers were removed and processed in the same manner as described for the Day 3 pepper collection to serve as a post-spray assessment of ovipositional ability and fly fertility. After fruit removal, two yellow bottom Multilure traps (Better World Manufacturing, Fresno, CA), each baited with a 300 ml solution of 8% Sol- ulys AST (Roquette America, Inc., Bridgeview, IL, http://www.roquette.com), 4% borax and 88% water, were added to each cage. Traps were hung from a wire strung across the top frame of the cage so that the trap was positioned surrounded by foliage and with foliage beneath the trap. On Day 9, the traps were serviced for recovery of any captured flies, visual counts were made of any untrapped live flies remaining in the cages, and the trial was terminated. Application rate of GF-120NF Fruit Fly Bait differed between bioassays. In Bioassay no. 1, a total of 12 ml of GF-120NF Fruit Fly Bait was applied to plants in the Treatment cage and 12 ml of GF-120 Fruit Fly Bait blank was applied to plants in the control cage, 2 ml (1.0 ml in each of two spots) on each plant. This bioassay was repeated. In Bioassay no. 2, the bait spray application rate was cut in half to 6.0 ml (one 1.0 ml spot per plant). Additionally, sucrose and water were added to both control and treatment cages to improve adult fly survivorship. The sucrose was provided by hanging a sugar cube (attached to an expanded paper clip with hot glue) on each plant in each cage. The water was provided by hanging a 2.5 cm diameter, 15 cm long cotton wick soaked in water from each plant in each cage. Plants were watered and wicks were rehydrated every 2 days. This bioassay was also repeated. For Bioassay no. 2, cage selection for treatment and control was reversed between replicates, with the treatment cage washed out upon completion of the first trial.

Statistical analyses
Significance of differences between control and treatment infestation rates (expressed as B. latifrons individuals per 100 g of peppers) in each bioassay replicate were analysed using a Wilcoxon two-sample test (Sokal and Rohlf 1981) with critical values of U obtained from Sokal and Rohlf (1969). This non-parametric (distribution-free) analysis is not based on an assumption of normality and was selected because post-spray infestation rates were all zeros, so did not meet the normal distribution assumption of parametric analyses such as t-tests or analysis of variance (ANOVA).

Results
Temperature and percentage relative humidity (%RH) were comparable in the cages among the replicates of each bioassay, with a slight trend for decreased temperature and increased RH over time [average temperature ± SEM = 20.6 ± 0.5°C (range = 19.5–21.9°C); average %RH ± SEM = 96.4 ± 0.6%RH (range = 95.3–97.9%RH)]. Average numbers and weights of peppers used per plant per replicate of each bioassay, numbers of B. latifrons recovered and recovery per 100 g of pepper is presented in Table 1 for fruits recovered both prior to bait spray application and after bait spray application. Also presented is the number of flies recovered in protein-baited traps and visually observed at the end of each of the bioassays. There was no significant difference in infestation rate of peppers collected before the application of the bait spray in control vs. treatment cages. This indicates that fly populations were comparable up until the time of bait spray application (Bioassay no. 1, replicate no. 1: U = 23, n1 = n2 = 6, P > 0.2; Bioassay no. 1, replicate no. 2: U = 23, n1 = n2 = 6, P > 0.2; Bioassay no. 2, replicate no. 1: U = 25, n1 = n2 = 6, P > 0.2; Bioassay no. 2, replicate no. 2: U = 25, n1 = n2 = 6, P > 0.2). However, after bait spray application, infestation of fruits was found only in the control cages in each replication of both of the bioassays. Infestation rates were significantly lower in the treatment cages relative to the control cages (Bioassay no. 1, replicate no. 1: U = 33, n1 = n2 = 6, P < 0.05; Bioassay no. 1, replicate no. 2: U = 33, n1 = n2 = 6, P < 0.05; Bioassay no. 2, replicate no. 1: U = 36, n1 = n2 = 6, P < 0.02; Bioassay no. 2, replicate no. 2: U = 36, n1 = n2 = 6, P < 0.02).

Although the same number of wild flies was added to both treatment and control cages, there were no flies in the treatment cage (based on both trapping results and visual observation) 6 days after the application of the bait spray in either replication of either bioassay. On the other hand, flies were both trapped and visually observed in control cages in both replications of each bioassay. Post-spray fly detections (both through trap catch and visual observation) in the control cages increased in the two replications of Bioassay no. 2 in which additional food and water had been added. However, there
continued to be no adult fly detection in the respective treatment cages.

**Discussion**

The results of this study provide evidence that GF-120NF Fruit Fly Bait should be effective in suppressing *B. latifrons* populations in the field. In each replication of each bioassay there was no infestation in peppers collected from treated plants, an infestation rate significantly less than in peppers collected from the associated control plants. Although replications were too few for statistical significance of differences in end-of-trial fly numbers between treatment and control cages in either Bioassay no. 1 or Bioassay no. 2, the end-of-trial fly numbers are supportive of the statistically significant differences found in infestation rates – absence of fly activity in the treatment cages. Because equal numbers of males and females were used in each replication of each bioassay, the results also show that males and females are comparably impacted by application of this bait spray, both having 100% mortality in each replication of each bioassay.

It is somewhat surprising that there was 100% mortality in the treatment cages in both replications of each bioassay. In outdoor cage tests one often observes that many flies will move to the screen walls, especially in the direction of light. This tendency could reduce exposure to the bait spray which was applied only to the underside of leaves on the host (pepper) plants. The flies on the screen, though, would still need to seek food and water and clearly the bait spray proved to be attractive to the flies. One unanswered question is how long an exposure to the bait spray was required to achieve 100% mortality. No infestation was found in peppers exposed to the cage environment for 2 days, starting 2 days after the bait spray was applied, and no adult flies were found 6 days after the bait spray was applied. It would be good to know if infestation could have been stopped if only half-day or 1 day was allowed before the remaining paper bags were removed, and also good to know how much earlier than 6 days the adult mortality reached 100%.

Although the cage trials reported here provided a good test of the effectiveness of GF-120NF Fruit Fly Bait against wild *B. latifrons* adults, there are additional issues of concern in field *B. latifrons* suppression efforts. Persistence of attractiveness of the bait will be one concern, both because the bait may be exposed to rain and because there will be continued adult recruitment from infested fruits – so adult emergence will be expected at times following bait spray applications. The relative responsiveness of different adult ages may also be a concern because a full range of adult ages would be expected, rather than all sexually mature adults as existed in the present study. However, for this issue, it is expected

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**Table 1** Infestation of Anaheim chili peppers in control and treatment cages both before and after application of GF-120 Fruit Fly Bait lacking spinosad (control cages) and GF-120NF Fruit Fly Bait (treatment cages)

<table>
<thead>
<tr>
<th>Bioassay no.</th>
<th>Replication no.</th>
<th>Timing</th>
<th>Treatment</th>
<th>Average no. fruit</th>
<th>Average fruit weight (g)</th>
<th>Average no. <em>B. latifrons</em> recovered</th>
<th>Average no. pupae per 100 g fruit</th>
<th>SEM</th>
<th>Number of flies recovered</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Pre-Spray</td>
<td>Control</td>
<td>7.8</td>
<td>11.3</td>
<td>44.2</td>
<td>54.5</td>
<td>9.2</td>
<td>n/a n/a 13 13</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Treatment</td>
<td>6.2</td>
<td>12.4</td>
<td>34.3</td>
<td>42.8</td>
<td>8.4</td>
<td>n/a n/a 25 25</td>
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<td></td>
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<td>Control</td>
<td>4.2</td>
<td>23.6</td>
<td>8.8</td>
<td>9.0</td>
<td>6.9</td>
<td>1.30 0 3 4</td>
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<td>Control</td>
<td>6.0</td>
<td>21.1</td>
<td>131.8</td>
<td>106.2</td>
<td>13.4</td>
<td>n/a n/a 55 55</td>
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<td></td>
<td></td>
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<td>33.6</td>
<td>33.3</td>
<td>21.6</td>
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<td>22.9</td>
<td>48.8</td>
<td>42.7</td>
<td>9.7</td>
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<td></td>
<td></td>
<td></td>
<td>Treatment</td>
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<td>37.2</td>
<td>27.8</td>
<td>5.4</td>
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<td>Control</td>
<td>4.3</td>
<td>37.9</td>
<td>31.3</td>
<td>42.3</td>
<td>23.4</td>
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<tr>
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<td>Treatment</td>
<td>4.3</td>
<td>25.9</td>
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<td>11.9</td>
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<td>84.3</td>
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<td>9.0</td>
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<td>31.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0 0 0 0</td>
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</tbody>
</table>

In Bioassay no. 1, replications 1 and 2, 12 ml of bait was applied (2 ml per plant), whereas in Bioassay no. 2, replications 1 and 2, only 6 ml was applied (1 ml per plant). No trapping was done at the end of the pre-spray period, indicated by ‘n/a’ in the appropriate cells of the table.
that younger adult flies would more readily feed on the bait because of the increased protein needs associated with egg development (Miller et al. 2004).

Another issue of concern is the optimal placement of the bait spray. In our cage study, vegetation consisted of only potted pepper plants placed on top of mowed field vegetation (grasses). Under normal field conditions involving either wild or cultivated host plants, additional trees and shrubs are likely to be present. It is recommended that the bait spray be applied directly to the underside of the leaves of the host plants as was done in this study. However, the extent to which non-host plants may be used as roosting hosts, as with melon fly, B. cucurbitae (Coquillett) (McQuate and Vargas 2007), and the effect of such on control using GF-120NF Fruit Fly Bait needs to be studied.

Even with the additional complications associated with field suppression activities, the data presented here are strongly suggestive that GF-120NF Fruit Fly Bait should be an effective B. latifrons suppression technique. It is good to know of a technique that is expected to be effective for B. latifrons population suppression, especially given the recent range expansion of this species. It is hoped that additional suppression capabilities can be improved for this species, including the development of a genetic sexing strain for use in sterile insect technique applications, the development of improved attractants and the development of biological control agents that are more effective than those currently established in Hawaii (Bokonon-Ganta et al. 2007).

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References


