Effects of spinosad-based fruit fly bait GF-120 on tephritid fruit fly and aphid parasitoids


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Received 7 March 2005; accepted 1 July 2005
Available online 22 August 2005

Abstract

The spinosad-based fruit fly bait GF-120 has recently been developed as a primary tool for the area-wide control and eradication of tephritid fruit flies. In this study, we assessed the direct contact toxicity of GF-120 to three major parasitoids of tephritids in Hawaii: Fopius arisanus (Sonan), Diachasmimorpha tryoni (Cameron), and Psyttalia fletcheri (Silvestri) (Hymenoptera: Braconidae), as well as one aphid parasitoid, Aphidius transcaspicus Telenga (Hymenoptera: Aphidiidae). All four parasitoid species were susceptible to GF-120. Males and females were equally susceptible to GF-120 for all species. The 24-h LC50 values for the opine braconid species were in a narrow range (8.3–17.5 ppm). The aphidiid appeared to be more susceptible than the opiniines, probably due to the stickiness of GF-120. We confirmed that adult F. arisanus (as a model species) do not feed directly on GF-120 either in the presence or the absence of honey and water resources. F. arisanus tasted, discriminated, and gave up GF-120 droplets after a brief (<1 s) mouth examination. Mortality following exposure to GF-120 resulted from close contact. Furthermore, we found that when female F. arisanus were allowed to freely forage on host coffee branches sprayed with droplets at the recommended field rate for use of GF-120 (80 ppm), treatment mortality was significantly higher than control mortality (sprayed with water), and also increased with exposure time. Although GF-120 appears to be the most judicious of reduced-risk fruit fly baits currently available, our results suggest that area-wide application of GF-120 needs to be carefully monitored in situations where release or conservation of parasitoids is a prime concern.

Keywords: Aphidius transcaspicus; Diachasmimorpha tryoni; Fopius arisanus; Fruit fly parasitoids; GF-120; Parasitoid feeding; Psyttalia fletcheri; Spinosad toxicity

1. Introduction

Spinosad, a mixture of spinosyns A and D derived from the naturally occurring soil actinomycete Saccharopolyspora spinosa Mertz and Yao (Sparks et al., 1998), has been classified as an environmentally and toxicologically reduced-risk insecticide (Cleveland et al., 2001; Copping and Menn, 2000). However, according to a recent review by Williams et al. (2003b), among 25 parasitoid species tested, 78% of the laboratory studies and 86% of the field studies reported that spinosad was moderately harmful or harmful to the parasitoids. Thus, the use of spinosad-based products should be evaluated carefully with respect to the need for biological control by augmentative release and/or conservation of parasitoids.

Biological control using parasitoids has been successful in suppression of the major tephritid pests, the Mediterranean fruit fly, Ceratitis capitata (Wiedemann), and...
the oriental fruit fly, *Bactrocera dorsalis* (Hendel), in Hawaii (Purcell, 1998). Among established parasitoids of tephritids in Hawaii, *Fopius arisanus* (Sonan) is the dominant species (Vargas et al., 2001; Wong and Ramadan, 1987), partly due to its competitive superiority (Wang and Messing, 2002, 2003a; Wang et al., 2003).

Other major fruit fly parasitoids include the braconids *Diaochasminomorpha tryoni* (Cameron) and *Pysttalia fletcheri* (Silvestri) (Hymenoptera: Braconidae). The former attacks *C. capitata* larvae, while the latter is the only effective parasitoid against larval melon fly, *Bactrocera cucurbitae* (Coquillet) (Purcell, 1998). Conservation of established parasitoids is important to natural control of tephritid pests because they have the ability to penetrate remote areas where other control techniques cannot be applied.

Fruit fly baiting is a common and important tool in tephritid control and eradication programs. Traditionally, the toxin mixed in tephritid baits has been the organophosphate malathion (Roessler, 1989), which is well known to have significant negative impacts on beneficial insects (Daane et al., 1990; Hoelmer and Dahlsten, 1993; Hoy and Dahlsten, 1984; Messing et al., 1995). Field tests demonstrated that spinosad-based baits have provided significant control of *C. capitata* and the Caribbean fruit fly, *Anastrepha ludens* (Leow), in Hawaii and Florida (Adán et al., 1996; Burns et al., 2001; King and Hennessey, 1996; Peck and McQuate, 2000). Laboratory tests showed that spinosad was extremely toxic to major tephritid pests in Hawaii when mixed with the most attractive protein Provesta (Stark et al., 2004b). Thus, GF-120 NF Naturalyte, a spinosad-based fruit fly bait (Dow AgroSciences LLC, Indianapolis, IN), has been developed as a replacement for malathion-based fruit fly baits. The product became commercially available in 2002 in the USA. In Hawaii, the current emphasis in area-wide tephritid management program is to use GF-120 baiting as a core management tool, followed by other management tactics including release of mass-reared parasitoids and sterile male flies, field sanitation, and male annihilation (Barry et al., 2003; Prokopy et al., 2003; Stark et al., 2004b; Vargas et al., 2001, 2002, 2003). GF-120 was chosen because it commonly attacks aphids in cultivated fruit and vegetable plantings where GF-120 may be sprayed for melon fly control. *A. transcaspicus* was originally introduced from Greece into California for biological control of the mealy plum aphid, *Hyalopterus pruni* (Geoffr.), on prunes (Mills, 2002), and was recently introduced from California into Hawaii for biological control of several invasive aphids (Messing, unpublished data). Second, we chose *F. arisanus* as a model species to carefully examine whether or not it will feed directly on GF-120 solution in the presence or absence of other resources (e.g., honey, water). Third, we determined the effect of GF-120 on *F. arisanus* when female wasps foraged freely on host coffee branches that were sprayed with droplets at the recommended field rate of GF-120.

2. Materials and methods

2.1. Parasitoids

The braconids *D. tryoni*, *F. arisanus*, and *P. fletcheri* were provided by the USDA-ARS U.S. Pacific Basin Agricultural Research Center at Honolulu, Hawaii, where they were mass-reared according to methods described by Wong and Ramadan (1992) and Bautista et al. (1999, 2000). Parasitized puparia were shipped from the rearing laboratory to the University of Hawaii at Manoa or the Kauai Agricultural Research Center (KARC) for different tests. A laboratory population of *A. transcaspicus*, initially started from cohorts imported from University of California at Berkeley, was maintained on the green peach *Myzus persicae* Sulzer at KARC. Parasitoid mummies were hand-carried to the University of Hawaii for bioassays.

2.2. Spinosad-based fruit fly bait (GF-120)

GF-120 NF Naturalyte fruit fly bait (Dow AgroSciences LLC, Indianapolis, IN) was used in this study. It contains 0.02% spinosad (AI) and 98.8% inert ingredients consisting of water, sugars, and attractants. The standard dilution recommended for field application for tephritids is 80 ppm (i.e., a mixture of 1 GF-120:1.5 water).

The bioassay of direct contact toxicity was conducted at the University of Hawaii at Manoa, Honolulu,
Hawaii, while caged feeding and exposure tests in cages were conducted at the KARC, Kauai, Hawaii.

2.3. Direct contact toxicity bioassay

Direct exposure of fresh GF-120 to the four parasitoids was conducted in 60 ml clear plastic vials under laboratory conditions (26 ± 2°C, 55–60% RH, 12L:12D). The stock GF-120 solution was serially diluted with distilled water to five different concentrations (5, 10, 20, 40, and 80 ppm). Distilled water was used as a control. Female and male wasps were separately tested for each species, except for P. fletcheri, where only female wasps were available for the test.

Prior to the tests, newly emerged adult male and female wasps were held together in screen cages (25 × 25 × 25 cm) for each opine species, and in small screen cages (9.5 × 10.5 × 13 cm) for the aphidiid. Water and honey were provided after eclosion. For all tests, we used 2- to 6-day-old braconids and 1- to 2-day-old A. transcaspicus from the holding cages.

Test procedures for each parasitoid species, sex, and treatment concentration were similar. For each replication, 10 wasps each were aspirated from a holding cage into a plastic vial and deprived of food and water for 12–24 h for braconid species and 6–12 h for A. transcaspicus prior to testing. A clean cotton wick (2 cm in length) was inserted into a vial cap and the wick was dipped into GF-120 solution until it was fully soaked. The cap with the soaked wick was then placed on the vial containing the parasitoids. After a 3-h exposure, the “wick” cap was replaced with a clean, screened cap, and a drop of undiluted honey (Spun, Premium Honey, USA) was added to the vial on the screened vial top to sustain the wasps. The number of dead wasps was recorded at 24 h following exposure. Individuals unable to stand and walk inside the vial were counted as dead. Tests for each treatment were replicated 10–30 times depending on the availability of wasps. Control mortalities of all parasitoid species were <10%.

2.4. Feeding by F. arisanus

To determine if adult F. arisanus is attracted to or directly feeds on GF-120, choice tests (in the presence of honey and water) and non-choice tests (with GF-120 only) were conducted under laboratory conditions (22 ± 3°C, 65 ± 10% RH, 3500 lux fluorescent cool white light with natural light accessible through window during the tests). Test methods were similar for both male and female wasps. Parasitized fly puparia from the rearing laboratory were placed individually in lidded plastic cups (45 ml). We used individuals that were 1–2 days old and had been deprived of food and water since their eclosion in the holding cups to maximize the possibility of feeding.

In the choice test, a single individual was provided with three different types of food: (a) GF-120 (80 ppm); (b) a 33% honey–water solution; and (c) pure distilled water. A fresh and clean coffee leaf was trimmed into a 5 cm diameter disc and placed into the center of a petri dish (9 cm diameter, 2 cm height). A 2–3 mm diameter droplet of GF-120, honey–water solution, and water were then placed on the leaf disc. Each droplet was applied randomly to one of three points of a 2 cm triangle. The cup holding a test wasp was inverted and positioned over the leaf disc to cover the triangle area. In the non-choice test, all three droplets consisted of the GF-120 solution. After the wasps contacted the coffee leaf in the choice test and started searching for food, we recorded the food type that the wasp first encountered, and whether or not the wasp fed on the first encounter, and its feeding time (if feeding behavior was observed). In non-choice tests, we recorded if a wasp fed on the GF-120 solution.

To compare the effects of feeding on adult longevity, a set of four treatments of wasps from the above tests were reared: (1) wasps that had only a single meal on honey; (2) wasps that had only a single meal on water; (3) wasps that had a first encounter with GF-120 in the choice tests and were continually provided with a droplet of GF-120 on a coffee leaf disc (2 cm diameter) for 24 h following the above tests; and (4) as a control, wasp that had been deprived of access to any food or water in the cups since emergence. Tests for each treatment were repeated about 30 times. To compare feeding times of wasps among various foods, extra observations were conducted to obtain a large enough sample size. Survival of the wasps was checked twice daily until they all died in each treatment. Individuals found dead and trapped in the GF-120 droplets were recorded separately.

2.5. Exposure of female F. arisanus to GF-120-treated coffee branches

This experiment was designed to determine the mortality of female F. arisanus when allowed to forage freely on coffee foliage sprayed with the recommended field rate of GF-120 (80 ppm). The experiment was conducted under the same laboratory conditions described above. We used 5- to 6-day-old female wasps that had been held with an approximately equal number of males in cages (25 × 25 × 25 cm). Water and honey were provided for the wasps since eclosion. Females used in this experiment were assumed to have mated and become sexually mature (Wang and Messing, 2003b).

A 10 cm long fresh coffee twig harboring about 10 intact coffee berries and 4–5 leaves (all others berries and leaves being removed) was inserted into a 200 ml vial filled with water. All the coffee twigs were first briefly exposed to sexually mature female C. capitata in a cage to
obtain host egg-infested coffee berries (which increase the parasitoids’ searching efforts, see Wang and Messing, 2003c), and then each coffee twig was sprayed with either GF-120 or water (as a control) using a 473 ml hand-sprayer. The droplets (1–2 mm in width) were applied over leaves (two sides) and berries. Each treated coffee twig was held for 30 min prior to being placed into a test Plexiglas screen cage (30 × 30 × 30 cm). Finally, a cohort of 20 female wasps was released into each cage by collecting the wasps into a vial, which was then placed upright in the cage to allow the wasps to fly/walk out. Dead individuals were removed and counted at 24 and 48 h following the exposure. Treatments and controls were replicated 10 times.

2.6. Data analysis

For the direct contact toxicity bioassay, concentration–mortality regressions for each species by sex were estimated with probit analysis (Finney, 1971) using the statistical software PriProbit (Throne et al., 1995). Differences in toxicity were considered significant when 95% fiducial limits (fl) did not overlap. Because mortalities among different treatment concentrations were not significantly different for the aphid parasitoid, the LC50 value for this species was not estimated, but the mortality data were analyzed with one-way ANOVA and Turkey HSD test for multiple comparisons (JMP 4.1, SAS, Cary, NC). The frequency of first encounter with different types of food by the parasitoid in the choice test was analyzed using the \( \chi^2 \) Goodness of Fit test (JMP4.1). Longevity of wasps feeding on different food types in the feeding experiment, and mortality between the treatment and control in the cage exposure test were also analyzed with ANOVA. All percentage data were arcsine transformed before being subjected to analysis of variance.

3. Results

3.1. Direct contact toxicity

Males and females of all three braconid species were susceptible to GF-120. The 24-h LC50 values and 95% fl resulting from 3-h exposure to GF-120 were in a narrow range (8.3–17.5 ppm), and overlapped between T. arisanus and P. fletcheri, while D. tryoni was slightly more susceptible than the other two species (Table 1). When these braconids were exposed to the highest GF-120 treatment concentration (i.e., the recommended field rate), 24 h mortality was >80%.

Aphidius transcaspicus was susceptible to all GF-120 concentrations tested (Females: \( F_{5.38} = 10.5, \quad P < 0.01 \), males: \( F_{5.38} = 10.8, \quad P < 0.01 \) (Table 2). However, mortalities among the four highest treatment concentrations in both male and females were not significantly different. In this test, about 20–30% of individuals were trapped in the GF-120 soaked cotton wick, while no opiines were trapped on the wick in the former tests. The small aphid parasitoid may be more vulnerable to the stickiness of GF-120 within constrained spaces.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>No. tested</th>
<th>Slope ± SE</th>
<th>LC50 (95% fl) ppm</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. arisanus</td>
<td>Female</td>
<td>980</td>
<td>1.53 ± 0.11</td>
<td>15.7 (13.7–17.8)</td>
<td>204.1</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1152</td>
<td>1.53 ± 0.10</td>
<td>13.4 (11.8–15.2)</td>
<td>218.9</td>
</tr>
<tr>
<td>D. tryoni</td>
<td>Female</td>
<td>1368</td>
<td>1.22 ± 0.09</td>
<td>8.3 (6.9–9.8)</td>
<td>179.9</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1014</td>
<td>1.48 ± 0.29</td>
<td>10.0 (3.0–17.2)</td>
<td>25.3</td>
</tr>
<tr>
<td>P. fletcheri</td>
<td>Female</td>
<td>671</td>
<td>1.40 ± 0.15</td>
<td>17.5 (14.3–21.2)</td>
<td>87.5</td>
</tr>
</tbody>
</table>

LC50 values were estimated at 24 h after tested wasps were exposed to GF-120 for 3 h.

3.2. Feeding and attractiveness to F. arisanus

Adult F. arisanus actively searched for food resources during this test. In most cases, wasps immediately went back up onto the cup after one meal of honey or water. However, when wasps first encounter with GF-120, they sometimes continued to search for food, but we counted only those wasps that had a single encounter with or feeding on one of the three food types. In choice tests, the frequency distributions of the first encounter with honey, GF-120, and water were 60, 23, and 17% in female F. arisanus, and 66, 18, and 16% in male F. arisanus, respectively. Thus, wasps found honey–water droplets more often than water or GF-120 in their first encounters in both female (\( \chi^2_{0.05.2} = 0.98, \quad n = 30, \quad P < 0.05 \)) and male wasps (\( \chi^2_{0.05.2} = 1.44, \quad n = 44, \quad P < 0.05 \)), suggesting that the wasps were significantly attracted to the honey. Upon discovery of the honey and water, both male and female wasps readily consumed them. Time spent ingesting honey (females: \( 119.9 \pm 8.9 \text{ s}, \quad n = 22; \quad \text{males: } 96.7 \pm 11.5, \quad n = 27 \) ) was significantly longer than time spent drinking water (females: \( 34.7 \pm 14.1, \quad n = 9; \quad \text{males: } 53.7 \pm 18.0, \quad n = 11 \) ) for both males (\( F_{1,37} = 4.12, \quad P < 0.05 \)) and females (\( F_{1,30} = 26.2, \quad p < 0.01 \)).

Virtually no direct feeding on GF-120 was observed by F. arisanus. In both choice and non-choice tests,
wasps that encountered GF-120 quickly left after a brief (<1 s) gustatory examination of the food. However, no strong repellence by GF-120 was observed. Thus, the wasps discriminated food only after a brief examination with mouthparts.

Feeding on or exposure to different food resources significantly affected the longevity of *F. arisanus* (females: $F_{1,107} = 54.4, P < 0.01$; males: $F_{1,114} = 55.3, P < 0.01$) (Table 3). Male and female wasps differed with regard to the effects of food supply on longevity. In females, one meal on honey solution significantly increased their longevity. Feeding on water did not significantly increase the females’ longevity when compared with those deprived of any resources. In contrast, male wasps’ longevity was not significantly different between honey and water-fed individuals, although the longevity of those that consumed honey or water was significantly longer than the wasps having no access to any resources. When GF-120 was supplied for the first 24 h, 42.9% of females and 82.1% of males died during the first 24 h. Mean longevity of the wasps in this treatment was significantly lower than any of the other three treatments for both sexes. Of the dead wasps, 31.6 and 37.5% of males and females, respectively, were trapped in the droplets of GF-120.

3.3. Exposure of *F. arisanus* to GF-120-treated coffee branch

Female *F. arisanus* were observed to land on GF-120-treated coffee branches in search of hosts, and searching on treated branches resulted in significantly higher mortality than those on untreated branches while the mortality increased with exposure time (24 h: $F_{1,19} = 14.2, P < 0.01$; 48 h: $F_{1,19} = 28.4, P < 0.01$) (Fig. 1).

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Female wasps</th>
<th>Male wasps</th>
</tr>
</thead>
<tbody>
<tr>
<td>One meal on honey solution</td>
<td>7.5 ± 0.31 a</td>
<td>5.5 ± 0.26 a</td>
</tr>
<tr>
<td>Water was supplied for the first day</td>
<td>6.1 ± 0.28 b</td>
<td>5.1 ± 0.25 a</td>
</tr>
<tr>
<td>No food was provided</td>
<td>5.3 ± 0.26 b</td>
<td>3.8 ± 0.25 b</td>
</tr>
<tr>
<td>GF-120 was provided for the first day</td>
<td>2.5 ± 0.27 c</td>
<td>1.3 ± 0.26 c</td>
</tr>
</tbody>
</table>

Values within each column followed by the different letters were significantly different (ANOVA, $P < 0.01$).

4. Discussion

Direct contact with fresh GF-120 was moderately harmful to all parasitoid species we tested, according to the toxicity ratings for laboratory tests by the International Organization for Biological Control (<30% mortality, harmless; 30–79% mortality, slightly harmful; 80–99% mortality, moderately harmless; and >99% mortality, harmful). In comparison with LC<sub>50</sub> values of feeding toxicity of spinosad to the three fruit fly species, *C. capitata*, *B. dorsalis*, and *B. cucurbitae* (<5 ppm) (Stark et al., 2004b), the three fruit fly parasitoids appeared less susceptible to GF-120 (see Table 1). Spinosad has been reported to be toxic to many Hymenopteran parasitoids (Haseeb et al., 2004; Jones et al., 2005; Penagos et al., 2005; Schneider et al., 2004; Williams and Price, 2004; Williams et al., 2003a,b). In some parasitoids (e.g.,
Diadegma insulaer (Cresson) and Trichogramma exiguum (Pinto and Planter), LC50 values of direct residue toxicity to adult wasps (0.3–3.3 ppm) were similar to the topical LC50 values of their lepidopteran host species (0.1–3 ppm) (Bret et al., 1997; Sparks et al., 1998). In general, small parasitoid species like Encarsia formosa, A. transcaspicus, and some egg parasitoids (e.g., Trichogramma) (e.g., Jones et al., 2005; Williams and Price, 2004) are more susceptible than large parasitoid species. In our study, using similar methods, we found that the aphid parasitoid was more susceptible than the opinines. However, the actual impact of insecticide toxicity on population levels would depend on life history traits such as intrinsic rate of increase (Stark et al., 2004a).

Our study confirmed that F. arisanus does not feed directly on GF-120. The parasitoid was significantly attracted to honey resources. Thus, ingestion appears to be an unlikely route of intoxication of spinosad to adult parasitoids and mortality may have resulted from direct contact. We observed that wasps often became trapped in the baits and discriminated against the unacceptable food of GF-120 through a brief mouth examination. The sticky consistency of fresh GF-120 is a potential threat particularly to small aphid parasitoids. Stark et al. (2004b) reported that fruit fly parasitoids appeared less susceptible to dry residual toxicity of spinosad; higher mortalities of adult F. arisanus and P. fletcheri were found only after exposure of these parasitoids to dry spinosad residues coated inside glass vials with concentrations >500 mg/liter. This suggests that the real threat of spinosad may be its fresh residue toxicity, rather than dry residue toxicity.

Direct contact toxicity of parasitoids with fresh GF-120 may depend to a large extent on the degree of confinement of individuals with the baits. Under the conditions of close confinement in small vials, exposure of female F. arisanus to the standard dilutions of GF-120 for 3 h caused 88.9% mortality 24 h after exposure. In contrast, when female F. arisanus were allowed to freely forage on coffee branches treated with the same rate of GF-120, a relatively lower mortality occurred after 24 h exposure (Fig. 1). Thus, in the field we perhaps expect a lower impact from direct contact toxicity of GF-120 to parasitoids. In Hawaii, Vargas et al. (2001) examined the effects of spinosad field applications on F. arisanus in coffee plantations on Kauai Island. They found that the number of F. arisanus recovered from malathion-baited plots was lower than from spinosad-baited plots during a two-week post-treatment period, suggesting reduced impact of spinosad bait on parasitoids compared to malathion bait, although it was unknown whether reductions of F. arisanus were due to decreases in host numbers or to morality of adult parasitoids in treated plot (as the number of host flies was also lower in the malathion plots than the spinosad plots, and percentage parasitism was not measured) (Vargas et al., 2001). In Florida, Burns et al. (2001) released Diachasmimorpha longicaudata, a larval fruit fly parasitoid, after a spray of spinosad-based baits in commercial orange groves, but due to the small numbers of recaptures of released parasitoids both in test and control plots and low replicates their data were insufficient to assess the non-target impact.

Further studies are needed to determine the effects of large-scale field applications GF-120 on fruit fly parasitoid populations, and on methods to improve its compatibility with the use of parasitoids. Because of its rapid decay (Cleveland et al., 2001) and low dry residue toxicity to parasitoids (Stark et al., 2004b), spinosad shows promise as an adjunct to augmentative release of parasitoids. Low dry residual toxicity allows the release of parasitoids relatively soon after application. However, we need to determine the contact toxicity of field-weathered residues, to determine at what point the contact toxicity of GF-120 decreases enough to recommended augmentative release of parasitoids in the field following the use of this product. This study also suggests that pre-feeding mass-reared fruit fly parasitoids with honey before release may not only increase their longevity, reproduction, and efficacy (Bautista et al., 2001; Wang and Messing, 2003b) but also reduce their risk of encountering toxic droplets of GF-120.

Acknowledgments

We thank Terri Moats for assistance, the USDA-ARS U.S. Pacific Basin Agricultural Research Center (PBARC) for kindly providing fruit fly parasitoids, and Nick Mills (University of California, Berkeley) for providing the aphid parasitoid. We also thank Eric Jang, Roger Vargas, Richard Kurashima, Dewayne Kawamoto, and Russell Ijima (PBARC) for their support of this study, and John Stark (Washington State University), Roger Vargas, and two anonymous reviewers for their useful comments on early versions of the manuscript. This research was supported by the USDA-ARS Grant # 5853208147 to R.H.M. and E.J.

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