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Lethal and sublethal effects of spinosad-based GF-120 bait on the tephritid parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae)

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Abstract

Experiments were performed to determine the likely compatibility of spinosad-based GF-120 applications and mass-releases of the braconid parasitoid *Diachasmimorpha longicaudata* (Ashmead) for control of tephritid fruit flies. Severe effects on *D. longicaudata* survival and reproduction in the laboratory contrasted with mild effects in semi-field studies. Topical application and contact with dried residues on plastic surfaces in the laboratory generally resulted in high mortality of wasps and significantly reduced survival times compared to controls. Survival times of both sexes were more severely affected when exposure to dried residues on mango leaves compared to wasps that were offered GF-120 mixed with honey. Brief (24 h) exposure to GF-120 in honey or dried residues on mango leaves did not affect total progeny production by wasps, whereas continuous exposure over a 10 d period resulted in reductions in female survival, progeny production and net fecundity ($\sum L_x M_x$) compared to honey-fed controls. During the rainy season, wasp survival times in field cages with young mango trees that had been sprayed with GF-120 or treated with GF-120 mixed with honey were only reduced by 1 or 2 days, respectively, compared to average 10.1 d survival of honey-fed controls. During the dry season, wasp mortality was not significantly affected by exposure to GF-120 residues on mango leaves that had been subjected to natural weathering in the field, compared to controls. Our results suggest that applications of GF-120 are unlikely to adversely influence the effectiveness of mass-releases of *D. longicaudata*. This prediction requires validation from comparative field studies on parasitoid populations in treated and untreated areas.

Keywords: Mango; Parasitoid reproduction; Spinosad bait spray; Survival; Tephritidae

1. Introduction

Tephritid fruit flies are currently controlled in Mexico and Central America by areawide applications of baits containing malathion or a naturally-derived insecticide spinosad (GF-120, Dow Agrosciences). Malathion continues to be used in Mexico and Central America due to its low cost, although the favorable toxicological and environmental characteristics of spinosad (USDA, 2001), means that it is likely to fully replace malathion for fruit fly control in the near future. Specifically, spinosad is a mixture of two macrolide lactones, spinosyns A and D, produced by fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (1990). Spinosad is classified by the U.S. Environmental Protection Agency as a reduced-risk material due to its low environmental persistence and very low toxicity to most vertebrates (Thompson and Hutchins, 1999). A review of laboratory and field studies indicated

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that spinosad can be applied without significantly harming populations of most predatory insects (Williams et al., 2003). However, spinosad was classified as moderately harmful or harmful in ~80% of studies involving parasitoid wasps. Parasitoids that survived exposure to sublethal doses were also far more likely to show adverse effects on longevity or reproductive capacity than predatory insects.

Programs of parasitoid augmentation are being undertaken in this region, mainly involving the mass-rearing and liberation of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). This wasp originated in the Indo-Pacific region where it parasitizes at least 14 species in the genus *Bactrocera* (Wharton and Gilstrap, 1983). The parasitoid has proved effective at reducing fruit fly populations in Florida (Sivinski et al., 1996), Mexico (Montoya et al., 2000) and several other countries in Central and South America (Ovruski et al., 2000). This species develops as a solitary kionobiont endoparasitoid of larvae and emerges from the host pupal stage.

Arial applications of GF-120 are now being performed over large areas of coffee plantations and fruit orchards in Central America (Enkerlin, 2005) and in fruit-growing areas of the United States, including Hawaii. GF-120 bait is based on hydrolyzed maize protein, invert sugars, small quantities of oil, gum, sorbates and other adjuvants, and 1% (wt/vol) ammonium acetate (Moreno and Mangan, 2003). The bait is formulated with 0.02% spinosad to a final concentration of 80 parts per million (ppm) of active ingredient (ppm a.i.). The ammonium ingredient is designed to attract fruit flies that often supplement their intake of nitrogen by feeding on bird feces (Aluja et al., 2000). Moreover, the ammonium component of GF-120 makes it less attractive to bees (Edwards et al., 2003), and unattractive to *Fopius arisanus* (Sonan), a parasitoid of tephritids (Wang et al., 2005).

Insect natural enemies present in crops that are subjected to insecticide applications will acquire toxicant by direct contact, by walking over surfaces that have toxicant residues, and by consuming contaminated food. Insects that do not die as a result of a pesticide dose can suffer sublethal effects that may reduce their effectiveness as natural enemies resulting in decreased pest control (Desneux et al., 2007). In the present study we employed a combination of laboratory and semi-field experiments to examine the likely impact of GF-120 applications on the survival, longevity, and reproductive capacity of *D. longicaudata*. The results provide useful insights into the likely compatibility of augmentative releases of parasitoids with areawide spraying programs involving the use of spinosad-based GF-120 bait.

2. Materials and methods

2.1. Insects, insecticide and field site

Parasitoids were obtained as parasitized pupae of *Anastrepha ludens* from the *D. longicaudata* mass production facility located in the Moscafrut plant, Metapa de Dominguez, Chiapas, Mexico. Prior to experiments, parasitoids were held in groups of approximately 750 insects in ventilated acrylic cages, 30 × 30 × 30 cm, containing a tube of water and a paper wick. All parasitoids were 5-d old at the start of laboratory and field experiments. All laboratory experiments were performed at 24 ± 2 °C, 60–80% humidity and a 12 h:12 h photoperiod. Field experiments were performed in a mixed mango (*Mangifera indica* L.) and sapodilla (*Manilkara zapota* L.) orchard located 1 km from the Moscafrut plant. The commercial formulation of spinosad, GF-120 naturalyte NF (Dow Agrosciences LLC, Indianapolis, IN) was prepared following manufacturer recommendations to give a final concentration of 80 ppm active ingredient (a.i.).

2.2. Topical application

Groups of 30 female parasitoids were individually treated with GF-120 in distilled water. Each parasitoid was gently held between thumb and forefinger while a 0.5 µl volume was carefully applied to the dorsal surface of the thorax using a micropipette. Control parasitoids were treated with water alone. Treated parasitoids were placed in an acrylic cage 30 × 30 × 30 cm and provided with a Petri dish containing tissue paper soaked in honey and a tube of water with a paper wick. Mortality was noted daily for 5 days post-application. Insects that did not move or respond to the touch of a toothpick were classified as dead. The experiment was performed four times.

To examine the influence of increased droplet volume and reduced spinosad concentration, a similar experiment was performed in which GF-120 was diluted to a concentration of 20, 40, and 80 ppm a.i. Groups of parasitoids were treated with a 2 µl drop of one concentration or a water control. Mortality was noted at 24 h intervals for 3 days post-application. The experiment was performed six times.

2.3. Contact with treated surfaces

Plastic tubes (8 cm diameter, 13 cm height) were lined with a cotton cloth that had been moistened with a solution of GF-120 containing 20, 40 or 80 ppm a.i. The cloth remained in position for 24 h at a temperature of 28 °C and was then removed. Control cloths were untreated. Tubes were left to dry completely for a further 24 h, whereupon groups of 10 female parasitoids were placed in each tube. The open end of the tube was sealed with fine nylon gauze and tissue paper soaked in honey was placed onto the gauze as a food source. Parasitoids were checked daily for mortality up to 10 days post-application. Each treatment was replicated 43 times.

2.4. Lethal and sublethal effects of contact and consumption

Groups of 50 male and 50 female parasitoids were placed in glass cages (30 × 30 × 30 cm) with one of the fol-

lowing treatments: (i) a drop of approximately 2 cm diameter of honey placed on the lid of a Petri dish, that was placed at the center of the base of the cage, and lined with tissue paper to prevent parasitoids becoming trapped by the honey, (ii) an identical treatment in which GF-120 (80 ppm a.i.) was prepared using 50% (vol/vol) of honey, (iii) a twig ~15 cm in length from a mango tree bearing 6–8 leaves that were sprayed to a point just prior to run-off with GF-120 solution 1 h prior to the start of the experiment. This was ample time to permit drying of spray residues under the warm conditions (>30 °C) of the mango orchard. The honey + GF-120 treatment was designed to imitate the possible consumption of GF-120 droplets deposited on aphid or scale insect honeydew-contaminated leaves. Each twig was placed in a 100 ml capacity tube of water with a perforated plastic cap, (iv) no food source. All cages contained a glass tube filled with water and with a moist filter paper wick. The mortality of each sex was recorded daily for 10 days. The experiment was performed 15 times.

In a separate experiment, 50 male and 50 female parasitoids were placed in glass cages and subjected, during a 24 h period, to one of the four treatments described above. Wasps were then transferred to cages containing a water source and a honey source (except the no food treatment). Each day for a 10 day period, 200 *A. ludens* third-instars were placed in the lid of a Petri dish containing larval diet and covered with a piece of organdy mesh held in placed by an elastic band (Wong and Ramadan, 1992). This is the usual parasitism device used to maintain the parasitoid colony and female parasitoids readily respond to the presence of host larvae in the dish. The oviposition device was placed on the floor at the center of the cage and parasitism was allowed to proceed for a period of two hours. After this time the dish was removed, larvae were separated from diet by gentle washing in a sieve, placed in plastic dishes (10 cm diameter × 5 cm height) with vermiculite and incubated at 26 ± 1 °C. The numbers and sex of parasitoids that emerged from each dish was noted daily. The experiment was performed twice. An identical experiment was performed in which wasps were continuously subjected to one of the four treatments for the 10 d duration of the experiment. The experiment involving continuous exposure was also performed twice.

2.5. Extended laboratory and field cage experiments

To determine the persistence of toxicity of GF-120 bait residues on mango leaves, an experiment was performed in the rainy season, from 23 July to 08 October 2004, during which time average (±SE) daily rainfall was 7.6 ± 1.5 mm. Field cages were constructed from nylon gauze with a 1 mm mesh size placed over a metallic frame of 1 m height × 0.3 m width × 1 m length in a mixed mango and sapodilla orchard. Young mango trees of approximately 1 m height, each planted in a black plastic bag of soil, were obtained from a nearby nursery. Trees were randomly assigned to one of the following treatments: (i) 15 drops of honey were placed on 80% of the leaves, (ii) a similar percentage of leaves were each treated with 15 drops of honey containing GF-120, (iii) the entire tree was sprayed to a point just prior to run-off with GF-120 in water using a manual back-pack sprayer and allowed to dry for 1 h prior to the start of the experiment. Both treatments involving GF-120 were performed using the recommended concentration of spinosad, but the quantity of product applied exceeded the coverage that would be expected following aerial application, for which the recommended rate of 4 L/ha of GF-120 usually results in a density of 60–80 droplets of 4–6 mm diameter per m^2. One treated tree was placed in each cage. Additional empty cages were used as controls. Four 125 ml capacity bottles of water with paper wicks were hung in every cage. Groups of 100 female and 100 male parasitoids were placed in each cage at 09.00 h on the day of the experiment. The number and sex of parasitoids that died was recorded daily over a 10 d period. The experiment was performed four times.

A second experiment was performed in December 2004, during the dry season, when fruits and flowers were absent and no rainfall occurred. Twenty-four trees of 4.5 m height were selected arbitrarily. Twenty-five drops (0.5 µl volume) of GF-120 were placed on five mango leaves located approximately 50 cm from the exterior canopy and 1.5 m above ground level. Treated leaves remained attached to the tree for periods of 1 h (day 0), 2, 5, 10 or 15 d prior to the experiment. On the final day, all leaves were cut-off, immediately transported to the laboratory and individually placed in tubes of water inside 30 × 30 × 30 cm acrylic cages. Groups of 30 female parasitoids were introduced into each cage. Control leaves were treated with water alone. Mortality was noted daily over a 5 d period. No additional food resource, other than water, was available to parasitoids inside acrylic cages. Four treatment trees and four control trees were sampled at each timepoint.

2.6. Statistical analyses

Results on topical applications of GF-120 were examined by fitting mixed-effects models with a compound symmetry correlation matrix structure, a binomial error structure and log or logit link functions specified, using the GENMOD procedure in SAS (SAS Institute Inc., 2001). Overdispersion was taken into account by scaling the error distribution. Repeated observations over time were considered as a within-subject factor with 3 or 5 levels. Mean times to death were estimated by fitting Weibull models to survival data from the experiments involving contact with treated surfaces or consumption of spinosad. Individuals alive at the end of each experiment were censored. Weibull analyses and cumulative mortality (binomially distributed data) in these experiments were analyzed in GLIM 4 (Generalized Linear Interactive Modeling)

(Numerical Algorithms Group, 1993) or S-Plus (Insightful Corporation, 2000). The suitability of Weibull models was determined by examination of Kaplan–Meier probability plots (Aitkin et al., 1989). Reproduction of wasps on *A. ludens* in laboratory cages was subjected to demographic analysis (Carey, 1993). The numbers of male and female progeny and the numbers of females alive on each day of the oviposition period were used to calculate the following parameters: (i) female life expectancy at the start of the experiment ($\sum L_x$) when wasps were 5-d old, (ii) gross fecundity ($\sum M_x$), being the hypothetical number of progeny produced by the longest-lived female of the cohort and, (iii) net fecundity ($\sum L_x M_x$), which is the average lifetime production of progeny per female. The numbers of insects that died in cages containing field-weathered spinosad residues on mango leaves were subjected to repeated measures analysis of variance in SPSS v. 10 (SPSS Inc., 2000).

3. Results

3.1. Topical application

In the first experiment on topical application, mortality in control parasitoids did not exceed 2.5% at any time (Fig. 1A), whereas mortality of parasitoids treated with GF-120 containing 80 ppm spinosad increased from 63% at 24 h post-treatment to 95% at 5 days post-treatment (treatment effect $Z = -6.39$, $P < 0.001$). In the second experiment, mortality of control insects did not exceed 1.7% at any time (Fig. 1B). A clear concentration-mortality response was observed in wasps treated with GF-120 containing 20, 40 or 80 ppm spinosad and overall mortality increased from 24 to 48 h ($Z = -7.28$, $P < 0.001$) and from 48 to 72 h post-treatment ($Z = -6.60$, $P < 0.001$).

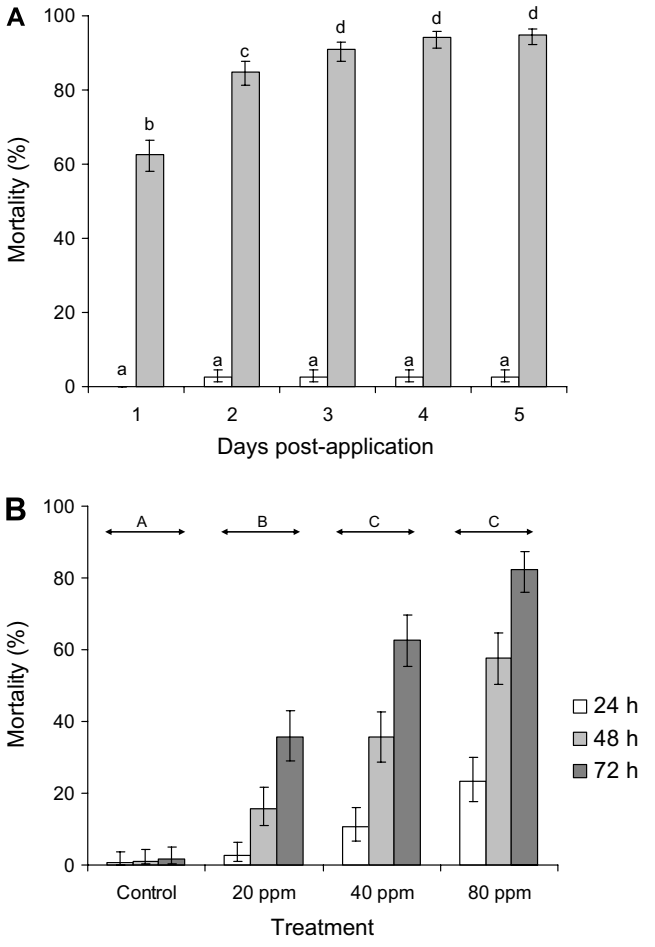

Fig. 1. Cumulative mortality of *Diachasmimorpha longicaudata* females that were subjected to topical application of (A) 0.5 μl drop of GF-120 containing 80 ppm spinosad (shaded bars) or a water control (open bars), or (B) 2 μl drop of GF-120 that had been diluted to a concentration of 20, 40 or 80 ppm spinosad or a water control. Columns headed by (A) different lower case letters or (B) treatment differences indicated by upper case letters above arrows were significantly different (mixed model analyses, $P < 0.05$). Vertical lines indicate SE.

3.2. Contact with treated surfaces

The 10 d mortality of parasitoids placed in plastic tubes that had been treated previously with cloths soaked in different concentrations of GF-120 increased significantly with treatment, from 13.1% in control insects to 98.5% in parasitoids held in tubes treated with 80 ppm a.i. (Fig. 2). Mean time to death estimates in control insects exceeded the 10 d period of the study and could not be reliably calculated. Mean time to death estimates of wasps that came into contact with spinosad-treated surfaces were concentration dependent, and ranged from 7.1 d (95% CI 6.7–7.5) in the 20 ppm a.i. treatment to 2.9 d (95% CI 2.8–3.1) in the 80 ppm a.i. treatment.

3.3. Lethal and sublethal effects of contact and consumption

The mean 10 d mortality of control wasps in cages with a source of honey was 11% in females and 39% in males (Fig. 3). Mortality increased significantly in both sexes when GF-120 was present in honey or as dried residues on the surfaces of mango leaves. Deprivation of food resulted in a similarly high prevalence of mortality (>95%) over the experimental period.

The mean time to death of control wasps exceeded the 10 d duration of the experiment and could not be reliably estimated. For the remaining treatments, estimated mean times to death values varied from 3.0 to 8.2 days and were invariably lower in males compared to female wasps (Fig. 3). Survival times for both sexes were highest in the honey + GF-120 treatment and lowest in the no food treatment for males and the dried residue treatment for females.

Demographic analysis indicated that total progeny production (males + females), average female survival time during the oviposition period ($\sum L_x$), gross fecundity ($\sum M_x$) and net fecundity ($\sum L_x M_x$) of *D. longicaudata* that had experienced a brief (24 h) exposure to GF-120 mixed with honey, or dried residues on mango leaves, and that were subsequently held in cages with a honey supply, was

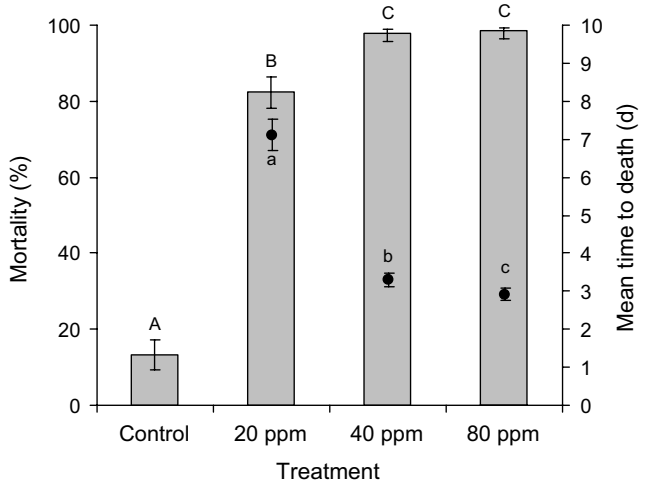

Fig. 2. Ten day mortality of *Diachasmimorpha longicaudata* females (shaded bars) placed in plastic cylinders containing dried residues of GF-120 that had been diluted to give concentrations of 20, 40 or 80 ppm a.i., or a water control. Bars headed by different upper case letters differed significantly (general linear model fitted in GLIM with binomial error structure, scale parameter = 1.37, $P < 0.05$); vertical lines indicate SE. Mean times to death (black points) were estimated by fitting survival curves to a Weibull distribution (shape parameter = 1.68). Points labeled with different lower case letters differed significantly (Weibull analysis, $P < 0.05$); vertical lines indicate 95% CI. Mean time to death of control insects exceeded 10 d and could not be estimated reliably.

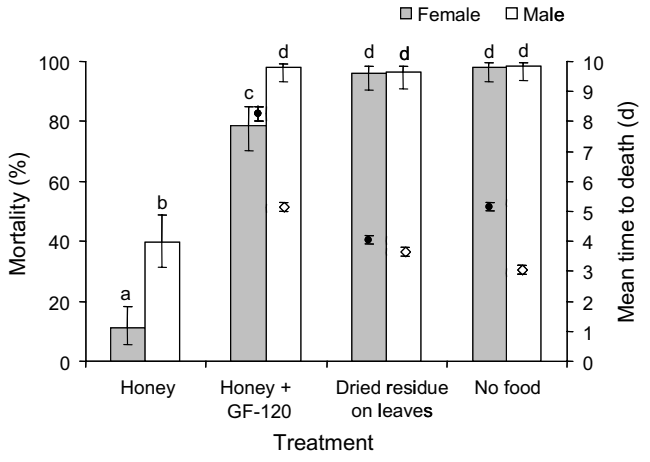

Fig. 3. Ten day mortality of *Diachasmimorpha longicaudata* females (shaded bars) and males (open bars) placed in cages containing no food source, honey alone or mixed with GF-120 (80 ppm a.i.), or mango leaves that had been sprayed with GF-120 and allowed to dry prior to the experiment. Bars headed by different upper case letters differed significantly (general linear model fitted in GLIM with binomial error structure, scale parameter = 5.40, $P < 0.05$); vertical lines indicate SE. Mean times to death (open and closed points) were estimated by fitting survival curves to a Weibull distribution (shape parameter = 1.93). Points labeled with different lower case letters differed significantly (Weibull analysis, $P < 0.05$); vertical lines indicate 95% CI. Mean time to death of control insects exceeded 10 d and could not be estimated reliably.

similar to that of control wasps that were exposed to honey alone (Table 1A). In contrast, progeny production, survival and net fecundity of wasps held in cages with no food source were markedly reduced compared to the other treatments. Progeny sex ratio (percentage of males) was significantly affected in wasps that had been briefly exposed to GF-120 in honey (28.3%) compared to the other treatments (23.6–25.9%) ($G = 16.3$, df = 3, $P = 0.001$).

Table 1
Demographic analysis of progeny production over a 10 d period by groups of *Diachasmimorpha longicaudata* held in cages with (A) 24 h exposure or (B) continuous exposure to GF-120 mixed with honey, dried GF-120 residues on mango leaves, honey alone or water alone

Treatment	Total progeny[a]	Female life expectancy ($\sum L_x$)[b]	Gross fecundity ($\sum M_x$)	Net fecundity ($\sum L_x M_x$)
A. 24 h exposure				
GF-120 in honey	2164	9.0	26.6	24.0
GF-120 spray	2409	7.6	34.3	25.9
Honey alone	2193	7.9	30.6	23.8
Water alone	1123	4.0	27.3	11.5
B. Continuous exposure				
GF-120 in honey	1097	4.8	18.1	12.3
GF-120 spray	632	2.0	23.5	7.4
Honey alone	2157	9.0	24.8	22.2
Water alone	1290	3.8	48.2	13.3

[a] Total number of male and female progeny produced by two cohorts of *D. longicaudata*.
[b] Female life expectancy in days at the start of the experiment when wasps were 5-d old.

Quite different results were obtained when parasitoids were continuously exposed to GF-120 over the experimental period. Continuous exposure to GF-120 in honey, or as dried residues on mango leaves, resulted in marked reductions in progeny production and decreased female survival times compared to control wasps exposed to honey alone (Table 1B). Progeny production and survival of wasps held in cages with no food source were similar to those of wasps from treatments involving continuous exposure to GF-120. As a consequence, net fecundity, defined as the product of survival and fecundity, was reduced by 45–66% in treatments involving GF-120, or no food source (40% reduction), compared to the honey-fed control treatment. The sex ratio of progeny (percentage of males) was also significantly affected by continuous exposure to GF-120 in honey (32.1%) or dried residues (40.1%) or in the absence of a food source (31.9%) compared to control wasps that had continuous access to honey (17.3%) ($G = 198$, df = 3, $P < 0.001$).

3.4. Extended laboratory and field cage experiments

During the rainy season, the estimated mean time to death of control wasps in field cages with a young mango tree that had been treated with drops of honey was 10.1 d (Fig. 4A); almost exactly equal to the duration of the experiment. Estimated times to death of wasps in cages with a mango tree that had been sprayed with GF-120 or GF-120 in honey were reduced by 1 or 2 days, respectively,

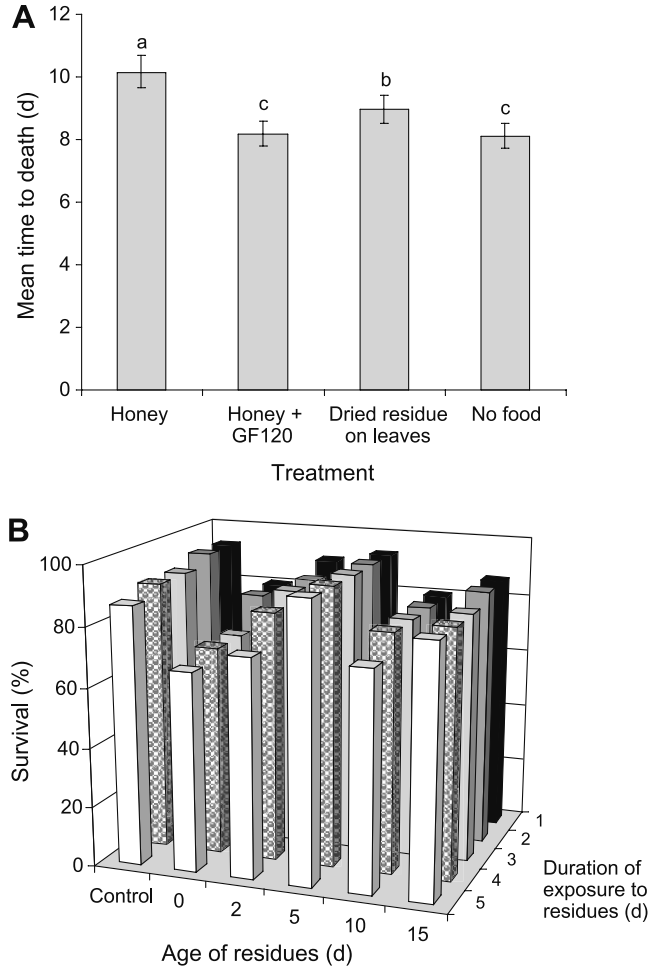

Fig. 4. (A) Mean time to death of *Diachasmimorpha longicaudata* females placed in field cages with a young mango tree treated with drops of honey alone (control) or honey mixed with GF-120, or dried GF-120 residues on leaves or no food source. Bars headed by different letters are significantly different (Weibull analysis, shape parameter $\alpha = 1.87$, $P < 0.05$); vertical lines indicate 95% CIs. The experiment was performed during the rainy season. (B) Survival of *Diachasmimorpha longicaudata* females placed in cages with a twig of mango leaves that had been sprayed with GF-120 (80 ppm a.i.) and allowed to degrade naturally in a mango orchard for between 1 h (day 0) and 15 days during the dry season. Control leaves were sprayed with water alone. Survival was noted daily for 5 days of the experiment.

compared to the control. Similarly, estimated time to death of wasps deprived of food was 2 days lower than the control.

In a second study performed during the dry season, control insects that were exposed to untreated mango twigs experienced consistently high survival (>87%) over the 5 d period during which they were held in cages containing treated foliage (Fig. 4B). Wasps held in cages with mango twigs that had been sprayed with GF-120 in the field at intervals of between 1 h (day 0) and 15 d prior to the experiment did not experience significantly reduced survival at any timepoint (within-subjects interaction age-of-residues × time $F = 0.744$, df $= 12.8, 46.2$, $P = 0.710$, Huynh-Feldt correction).

4. Discussion

Laboratory studies in which drops of GF-120 were applied directly to *D. longicaudata* indicated that this product is toxic by contact at the recommended application rate of 80 ppm spinosad. Wasp mortality was greater than 80% at 3 d post-application in both experiments. Droplet volume (0.5 vs. 2 μl) was less influential than concentration of toxicant. However, the mortality observed at 24–48 h post-application in wasps treated with the larger volume of GF-120 was lower than observed in conspecifics treated with a smaller volume of this product, possibly indicating that wasps treated with large volume drops engaged in more thorough cleaning activity than those treated with small volume drops, although observational data are required to support this hypothesis.

Walking over treated surfaces resulted in high levels of mortality and greatly reduced survival times in the laboratory, compared to control insects. It is important to note that the speed of kill of spinosad depends on the dose, the route of is acquisition of the toxicant and the target species. Fruit flies are high sensitive to spinosad and die within hours of ingesting GF-120 (Wang and Messing, 2006), whereas in parasitoid species, the mortality response may occur several days after the acquisition of a lethal dose (Mason et al., 2002; Williams et al., 2003; Penagos et al., 2005). Consequently, all the studies reported here involved evaluations over periods of 3–10 days.

Laboratory comparison of toxicity by exposure to GF-120 in mixtures with honey and contact with dried residues on mango leaves were consistent with the results of the topical application and treated surfaces experiments. Both routes of exposure resulted in a high prevalence of mortality and reduced survival times in both sexes of the parasitoid. Parasitoids were rarely observed feeding on mixtures of GF-120 and honey, although this was not quantified, suggesting that one or more components of the bait were unattractive to the parasitoid. Generally, the magnitude of the observed effects was similar to those of food deprivation.

Comparative studies on spinosad toxicity by ingestion and contact with treated surfaces have reported that, at field recommended rates, both routes of toxicant acquisition result in a very high prevalence of mortality for both a fruit fly parasitoid, *Pysttalia* (*Opius*) *concolor* (Szépligeti) (Viñuela et al., 2001), and parasitoids of other insect orders (Haseeb et al., 2004; Williams III and Price, 2004; Takahashi et al., 2005). Similarly, most studies involving topical application have reported that parasitoids are highly sensitive to spinosad when applied directly to the adult stage (Tillman and Mulrooney, 2000; Viñuela et al., 2001), or to parasitized hosts from which parasitoids subsequently emerge (Suh et al., 2000; Consoli et al., 2001; Mason et al., 2002; Schneider et al., 2004; Hossain and Poehling, 2006). In contrast, spinosad is not volatile and exposure to the fumes of this product had no significant effect on the mortality of two highly sensitive species of egg parasitoids (Williams III and Price, 2004).

In the present study, the effects of GF-120 treatment on the reproduction of *D. longicaudata* were dependent on the duration of exposure to the product. Brief exposure (24 h) to GF-120 in honey or dried residues on leaves had no discernable effects on the total number of progeny produced or net fecundity ($\sum L_x M_x$), and only a minor influence on progeny sex ratio in the treatment involving GF-120 in honey. In contrast, female survival was reduced in treatments involving continuous exposure to GF-120 resulting in reduced net fecundity values and lower progeny production compared to honey-fed control wasps. Continuous exposure to GF-120 also resulted in a significant increase in the proportion of male progeny, compared to control insects. The influence of spinosad on parasitoid reproduction has received little attention; treatment of hosts resulted in greatly reduced progeny production in a trichogrammatid (Consoli et al., 2001) and a pteromalid (Elzen et al., 2000). Treatment of parasitized hosts did not affect the sex ratio of *Trichogramma exiguum* Pinto and Platner that emerged from treated eggs (Suh et al., 2000). Adult males of the tephritid parasitoid, *F. arisanus,* and the pteromalid parasitoid of boll weevil larvae, *Catolaccus grandis* (Burks), were significantly more susceptible to spinosad treatment than conspecific females (Elzen et al., 2000; Wang et al., 2005).

Studies of the toxicity of residues that have been subjected to natural processes of degradation in the field usually conclude that spinosad degrades rapidly when assayed against a wide variety of natural enemies. Consequently, a low prevalence of mortality is normally observed at 3–7 days post-application on a variety of crops, including cotton (Suh et al., 2000; Tillman and Mulrooney, 2000), maize (Scholz and Zalucki, 2000; Penagos et al., 2005), citrus (Bernardo and Viggiani, 2000), and cabbage (Haseeb et al., 2004). However, one study has suggested that field persistence in cotton may be extended during periods of particularly dry weather (Williams III et al., 2003). Similarly, residues on crops grown under glass or plastic are protected from rainfall and ultraviolet radiation, such that the interval between application and the release of parasitoid biocontrol agents should take into account the extended persistence of spinosad on covered crops to ensure parasitoid effectiveness (Miles and Dutton, 2000). Indeed, GF-120 applied to small grapefruit trees in a greenhouse remained highly toxic to tephritid flies over a 5 week period (Mangan et al., 2006).

The results of the present study were in good agreement with most of the published findings regarding spinosad toxicity to parasitoid wasps. Importantly, the mortality responses and survival times of *D. longicaudata* that ingested or came into contact with dried spinosad residues in the laboratory were in marked contrast with the results of the field and semi-field studies performed during both rainy and dry seasons. It appears that under field conditions, *D. longicaudata* adults can avoid consumption or prolonged contact with spinosad residues. Indeed, direct observations of the feeding behavior of two tephritid parasitoids, *F. arisanus* and *Pysttalia fletcheri* (Silvestri), have indicated that these wasps do not feed on drops of protein baits, such as GF-120, but may briefly taste the bait before rejecting it (Vargas et al., 2002; Stark et al., 2004; Wang et al., 2005). Our findings also find support from field studies by Vargas et al. (2001) and McQuate et al. (2005) who observed marked reductions in fruit fly populations in areas sprayed with GF-120 that were accompanied by little change in the percentage of parasitism by the dominant species of parasitoid, *F. arisanus* in Hawaii. It is important to note however, that little work has been performed on the impact of GF-120 applications on parasitoids that attack non-tephritid hosts. Laboratory studies have reported high mortality in several parasitoid species that are common in areas subjected to GF-120 applications (Michaud, 2003; Wang et al., 2005). The potential of GF-120 applications to disrupt the biological control of secondary pests requires additional study, although preliminary findings on biocontrol of homopteran and mite pests in Texas have indicated little cause for concern (Thomas and Mangan, 2005). The compatibility of spinosad with programs involving the mass-release of hymenopteran parasitoids may be further improved by selecting for spinosad resistance during the parasitoid rearing process (Liu et al., 2007).

We conclude that the effects of contact or consumption of spinosad GF-120 bait on the survival and reproduction of *D. longicaudata* observed in the laboratory were generally severe, whereas the results from field cages, or exposure to field-weathered residues on mango leaves, were invariably minor. As such, it appears unlikely that current IPM programs involving mass-releases of *D. longicaudata* in Mexico would be disrupted by GF-120 sprays, particularly when a period of several days has elapsed between spraying and parasitoid releases. Temporal separation in the use of GF-120 and *D. longicaudata* is largely determined by the phenology of the host plant as GF-120 sprays are applied when fruits reach three-quarters ripeness and become suitable for oviposition by adult tephritids, whereas parasitoid releases are targeted at late-stage larval populations in fully ripe and fallen fruit. This prediction requires validation from comparative field studies on the reproduction and survival of *D. longicaudata* populations and natural enemies of secondary pest species in sprayed and unsprayed areas.

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