# Protection of Fruit Against Infestation by Apple Maggot and Blueberry Maggot (Diptera: Tephritidae) Using Compounds Containing Spinosad

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**ABSTRACT** Two insecticide formulations containing the naturalyte insecticide spinosad, GF-120 Fruit Fly Bait and SpinTor 2 SC, were compared for control of apple maggot, *Rhagoletis pomonella* (Walsh), and blueberry maggot, *Rhagoletis mendax* Curran. In 2002 and 2003, larval infestation in blueberries and apples was significantly lower in plots treated with GF-120 (spinosad bait) or SpinTor than in untreated control plots. Fruit fly infestation in apples was reduced by 67% in 2002 after weekly application of GF-120 for 6 wk. Six weeks of GF-120 treatment reduced infestation in blueberries by 85% in 2002 and 98% in 2003. Plots treated weekly with the bait component of GF-120 for 6 wk had significantly higher infestation of blueberry maggot larvae compared with untreated plots in 2002. Observations of wild *R. mendax* flies revealed that similar numbers of flies landed on blueberry foliage treated with spinosad bait, the bait component alone, or water droplets. However, flies on spinosad bait and bait treated plants spent significantly more time within 5 cm of the treatment droplets compared with control (water) droplets. Overall, the results demonstrate a high degree of efficacy of baited spinosad formulations against these key pests of temperate fruit and suggest that GF-120 is an arrestant for foraging flies.

**KEY WORDS** *Rhagoletis pomonella*, *Rhagoletis mendax*, bait sprays, insect control, behavior

THE APPLE MAGGOT, *Rhagoletis pomonella* (Walsh), and the blueberry maggot, *Rhagoletis mendax* Curran, are among the most important late-season pests of apples, *Malus domestica* L., and highbush blueberries, *Vaccinium corymbosum* L., respectively, in the United States and Canada (Bush 1966, Howitt 1993). Infestation of the crop by these frugivores renders it unmarketable in regions with quarantine restrictions for *R. mendax* and *R. pomonella.* Fruit destined for regions certified as being free of these pests is inspected and detection of one infested fruit leads to rejection of the entire load. Currently, organophosphates such as phosmet, malathion, and azinphosmethyl are the most widely used insecticides in apples and blueberries for control of *Rhagoletis* fruit flies (Wise et al. 2003). However, increased restrictions on the use of these broad-spectrum insecticides imposed by the U.S. Environmental Protection Agency in response to the 1996 Food Quality Protection Act (Anonymous 1996) will provide a challenge for successful insect management in these crops. In particular, extensions of the reentry and preharvest intervals for the most efficacious insecticides against fruit flies will restrict their utility for the critical period of control just before harvest.

Some new insecticide chemistries that have recently been registered show promise as alternative controls for fruit flies, and others are being developed that should be registered over the next few years (Wise and Gut 2002, Wise et al. 2002). Spinosad is a naturalyte insecticide consisting of two compounds derived from the actinomycete *Saccharopolyspora spinosa,* spinosyns A and D (Mertz and Yao 1990). Baited formulations of this naturally derived insecticide have been effective in controlling populations of several tropical fruit fly species, including Caribbean fruit fly, *Anastrepha suspensa* (Loew) (King and Hennessey 1996, Burns et al. 2001); Mexican fruit fly, *Anastrepha ludens* (Loew) (Moreno and Mangan 2002); and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Peck and McQuate 2000; Vargas et al. 2001, 2002). Bait sprays at concentrations as low as 1 ppm spinosad have provided significant fruit fly control (King and Hennessey 1996, Peck and McQuate 2000).

Spinosad used alone or in combination with bait has received much less attention as a possible control for temperate species of fruit flies. Smith (1999, 2000) reported control of *R. indifferens* in small-plot trials by using spinosad (Success, Dow AgroSciences, Indianapolis, IN) alone or with horticultural mineral oil. Two formulations of spinosad have recently been registered in the United States for use in blueberry and apple against *R. mendax* and *R. pomonella,* respectively, despite relatively little efficacy data against *Rhagoletis* fruit pests (Liburd et al. 2003, Reissig 2003). Spinosad was registered for use in apples and blueberries in 2002 as a foliar spray formulation (Success or SpinTor, Dow AgroSciences). GF-120 Fruit Fly Bait (Dow AgroSciences) is a baited formulation of spinosad that was first available for commercial use in apples and blueberries during 2003.

The lethal action of spinosad against *Rhagoletis* fruit flies occurs primarily through ingestion; there is little or no contact activity (Mangan and Moreno 1995, Vargas et al. 2002). Feeding in adult *R. pomonella* is greatest during the first week after emergence but decreases thereafter (Webster et al. 1979), so preventing larval infestation by using spinosad is likely to require novel approaches that entice the fly to consume a lethal dose of the toxicant. Optimizing the use of baited insecticides will require a better understanding of how *Rhagoletis* flies respond to various formulations under natural field conditions. Specifically, parameters such as duration of efficacy and attractiveness to flies will need to be investigated. Although efficacy of baited spinosad has been demonstrated in many systems (see above), in greenhouse assays, Prokopy et al. (2003) showed attractiveness of GF-120 decreased 50% by 5 h postapplication, suggesting the need for frequent reapplication.

The specific objectives of the current study were to 1) determine the effectiveness of GF-120 Fruit Fly Bait in controlling two temperate fruit fly species, *R. pomonella* and *R. mendax*, and 2) quantify the behavioral response of *R. mendax* flies to GF-120 under field conditions.

## Materials and Methods

**Experimental Sites.** Research was conducted in the summers of 2002 and 2003 in apple orchards at Michigan State University's Trevor Nichols Research Complex (Fennville, MI) and in a noncommercial highbush blueberry planting (Holland, MI). Field sites were chosen because of known histories of moderate-to-high infestations of *R. pomonella* or *R. mendax* and the lack of insecticide applications for at least 2 yr.

**Spinosad Sprays in Apples.** In 2002, experiments were conducted in a 1.0-ha apple orchard planted on a 3.0 m within-row by 5.5 m between-row tree spacing, with treatments applied to 12.4 by 22-m (0.03-ha) plots. Experiments in 2003 were conducted in 39.2 by 55.8-m (0.08-ha) plots in the same orchard and in an adjacent 1.3-ha orchard planted on a 2.4 by 5.5-m tree spacing. The 2002 experiment consisted of the following four treatments: 1) GF-120 Fruit Fly Bait (spinosad bait), 2) SpinTor 2 SC (unbaited spinosad), 3) bait (which includes all compounds in GF-120 except spinosad), and 4) untreated (control). Bait without the insecticide incorporated could not be obtained from the manufacturer in sufficient quantity during 2003; therefore, only three treatments were compared. Experiments in both years were arranged in a randomized complete block design with four replicates and at least a one-row buffer between plots.

Initial applications of all treatments were made 1 wk after the first *R. pomonella* fly was captured on Pherocon AM traps (Great Lakes IPM, Vestaburg, MI), on 11 July in 2002 and 15 July in 2003. An 80 ppm aqueous mixture of spinosad bait or bait [1:1.5 (vol:vol)] was applied at 2.4 liters/ha by using a MeterJet spray gun (model no. 23624-30L, Spraying Systems Co., Wheaton, IL). The handgun produced droplets of ≈4–6 mm diameter. SpinTor was applied at the recommended rate of 21.4 g [AI]/378 liters (8 oz in 100 gal/acre) by using an airblast sprayer (model no. CP3000, John Bean Sprayers, LaGrange, GA, in 2002; model no. 1029, FMC Corp., Lakeland, FL, in 2003). Spinosad bait and bait were applied weekly until harvest for a total of six applications, and SpinTor was applied every 2 wk for a total of three applications.

The relative abundance of flies in each plot was estimated by captures on unbaited red sphere traps (Great Lakes IPM) placed at the center of each plot. One trap per plot was hung ≈2.0 m above the ground, and all foliage within 0.5 m of the trap was cleared away (Drummond et al. 1984). Once per week, captured flies were counted and removed from monitoring traps.

Fruit infestation evaluations were conducted at the end of each growing season to determine the effectiveness of control for each treatment (12 September in 2002 and 2 September in 2003). In total, 100 apples per plot were randomly picked from the innermost four trees in 2002. In 2003, 250 apples were randomly picked from the innermost 10 trees. Fruit samples from each plot were kept separate and placed on wire hardware mesh suspended above trays containing fine vermiculite, allowing mature larvae exiting fruit to drop into the vermiculite medium and pupate. After 4 wk, the vermiculite was sifted for fly puparia, which were counted to determine the level of infestation per plot.

Effects of treatments on mean fly catches in traps throughout the season and mean larval infestation in fruit were determined by analysis of variance (ANOVA) followed by means separation by using Fisher's protected least significant difference (LSD) procedure ($\alpha = 0.05$) (PROC GLM, SAS Institute 1998). Before analysis, fly capture data were normalized by square-root transformation $[(x + 0.5)^{1/2}]$.

**Spinosad Sprays in Blueberries.** Experiments were conducted in a 1.8-ha highbush blueberry field planted at 2.7 by 3.7-m spacing, with treatments applied to 16.2 by 37-m (0.06-ha) plots in 2002 and 16.2 by 37.4-m (0.08-ha) plots in 2003. Treatments in 2002 and 2003 were the same as those tested in apple with the exception of SpinTor, which was applied at the lower registered blueberry rate of 14.2 g [AI]/378 liters (6 oz in 100 gal/acre). As in apples, bait was not available for the 2003 experiment. Four and five replicates of each treatment were used in 2002 and 2003, respectively, with treatments arranged in a randomized block design and plots separated by at least one row of bushes. All treatments were applied after the first fly caught on monitoring traps (8 July in 2002 and 12 July in 2003). Spinosad bait and bait were applied each week until harvest for a total of six applications as described previously. SpinTor was applied every 2 wk for a total of three applications.

Monitoring of *R. mendax* adults was conducted using Pherocon AM traps hung in a V-orientation $\approx$15 cm below the uppermost portion of the bush, the optimal position for capturing sexually mature *R. mendax* (Liburd et al. 2000). Each week, monitoring traps (one per plot) were checked for *R. mendax* flies, which were counted and removed.

Assessment of larval infestation was conducted in a manner similar to that described for apples. Samples of 1000 blueberries per plot were harvested on 24 August 2002 and 28 August 2003. Fruit were held for 1 mo before puparia were counted. Numbers of adult blueberry maggot flies captured on traps and larval infestation within treatments were compared using the methods described above for *R. pomonella.*

**Field Observations of *R. mendax*.** The first objective of the behavioral component of this study was to compare the number and duration of *R. mendax* visits to plants treated with 1) spinosad bait, 2) bait only, or 3) water (control). The second objective was to quantify the behavioral interactions of *R. mendax* with the GF-120 Fruit Fly Bait formulated with or without insecticide. All observations were conducted in a 1.8-ha unsprayed *V. corymbosum* ('Jersey') field in Douglas, MI. This planting was chosen because it historically harbors a high population of *R. mendax* (Stelinski and Liburd 2001). The experiment was conducted using a randomized complete block design with three replicates of three treatments: 1) spinosad bait, 2) bait, and 3) water as a control, with each replicate consisting of one treated plant. Treatments were applied at the beginning of each week for 4 wk. Five 10-$\mu$l droplets (2–3 mm in diameter, according to label recommendation) of each treatment were applied to each plant on the top surface of leaves spaced at least 30 cm apart. Observations of wild flies were conducted daily for 5 d posttreatment between 0800 and 1300 hours. During the observations, air temperature was $\approx$21–27°C. Investigators stood $\approx$0.5 m away from a treated bush and observed the plant for 20 min or until an observed fly left the plant. Data were collected by recording observations into handheld microcassette audio recorders (model no. 3-5375A, GE, Westminster, CO). The behaviors recorded were 1) number and duration of fly visits to blueberry plants, 2) number and duration(s) of feeding events on treatment droplets, and 3) duration of time spent within proximity (5 cm) of the nearest treatment droplet. The effects of treatments on mean numbers of *R. mendax* observed visiting blueberry bushes were determined using ANOVA followed by means separation by using Fisher's protected LSD procedure ($\alpha = 0.05$) (PROC GLM, SAS Institute 1998). Before analysis, data were normalized by square-root transformation $[(x + 0.5)^{1/2}]$. A two-sided sign test (BINOMDIST, Microsoft Corporation, Redmond, WA) was used to determine whether the number of flies observed on blueberry bushes varied over time after treatments, where Y = (flies observed on days 0–2) –(flies observed on days 3–4) (Sokal and Rohlf 1981). The probability ($P = 0.05$) of no difference in response to treatment over time (mean $Y = 0$) versus a change in response (mean $Y \neq 0$) was determined.

**Table 1. Effect of different spinosad formulations on mean (± SEM) apple and blueberry infestation by the apple maggot, *R. pomonella***

| Treatment | % infested apples | | % infested blueberries | |
|---|---|---|---|---|
| | 2002 | 2003 | 2002 | 2003 |
| Spinosad bait | 0.9 ± 0.3a | 0.4 ± 0.1b | 0.5 ± 0.3a | 0.1 ± 0.03a |
| SpinTor | 0.6 ± 0.2a | 0.2 ± 0.1b | 0.6 ± 0.2a | 0.3 ± 0.1a |
| Bait | 2.9 ± 0.0b | – | 2.5 ± 0.6b | – |
| Untreated (control) | 1.0 ± 0.4a | 1.2 ± 0.1a | 3.3 ± 0.5b | 4.4 ± 0.9b |

Means within each column followed by the same letter are not significantly different, ($P > 0.05$, Fisher's Protected LSD Test). Data were subjected to arcsine transformation before analysis.

Untransformed values are shown.

## Results

**Spinosad Sprays in Apples.** In 2002, mean captures of adult apple maggot flies on sphere traps in spinosad bait (1.9 ± 0.5), SpinTor (2.7 ± 0.6), and bait-treated plots (2.9 ± 0.6) were not significantly different ($F = 1.44$; df = 3, 9; $P > 0.05$) from captures on sphere traps in untreated plots (3.7 ± 0.8). Similarly, the number of flies captured in 2003 on sphere traps in spinosad bait (11.6 ± 2.5), SpinTor (11.5 ± 3.0), and untreated (19.3 ± 3.6) plots were not significantly different ($F = 0.44$; df = 2, 6; $P > 0.05$).

In 2002, larval infestation of apples was significantly lower ($F = 3.4$; df = 3, 9; $P = 0.049$) in the untreated, SpinTor, and spinosad bait plots compared with plots treated with bait (Table 1). There were no significant differences in mean apple infestation levels among the untreated, SpinTor, and spinosad bait plots. In 2003, the percentage of apple infestation by *R. pomonella* was significantly lower in GF-120 and SpinTor plots compared with untreated plots (Table 1) ($F = 7.16$; df = 2, 8; $P = 0.02$).

**Spinosad Sprays in Blueberries.** In 2002, captures of adult flies on Pherocon AM traps hung in spinosad bait (0.68 ± 0.26), bait (1.46 ± 0.38), SpinTor (1.43 ± 0.43), and untreated (1.68 ± 0.29) plots were not significantly different ($F = 2.74$, df = 3, 9, $P > 0.05$). In 2003, there were no significant differences in the numbers of flies captured on traps among any treatments ($F = 2.71$; df = 2, 8; $P = 0.3$).

In 2002, infestation of blueberries in plots treated with bait was not significantly different from that in untreated plots (Table 1). In both 2002 and 2003, plots treated with spinosad bait and SpinTor had significantly lower percentages of infested blueberries compared with untreated plots (2002: $F = 4.43$; df = 3, 9; $P = 0.02$; 2003: $F = 19.84$; df = 2, 8; $P = 0.007$) (Table 2).

**Field Observations of *R. mendax* Responding to GF-120.** There were no significant differences in the mean numbers of adult *R. mendax* observed visiting spinosad bait, bait, or untreated blueberry bushes (Table 2). For all treatments, the mean numbers of flies visiting bushes did not differ between days 0–2

**Table 2. Number (mean ± SEM) of *R. mendax* flies observed per week on blueberry plants treated with spinosad bait, bait, or water droplets and the duration (mean ± SEM) spent within 5 cm of the droplets, data collected 14 July–08 Aug. 2003**

| Treatment | Number of flies observed | Duration within 5 cm of droplet(s) |
|---|---|---|
| Spinosad bait | 8.8 ± 1.8a | 598.0 ± 175.8a |
| Bait | 11.8 ± 0.9a | 381.3 ± 141.1a |
| Water (control) | 8.0 ± 1.2a | 53.6 ± 38.0b |

Means within each column followed by the same letter are not significantly different ($P > 0.05$, Fisher's Protected LSD Test).

and days 3–4 (two-sided sign test, $P = 0.286$; $n = 22$). The mean duration of fly presence within 5 cm of spinosad bait and bait treatment droplets was significantly greater than the mean duration spent within the same distance of control (water) droplets ($F = 3.9$; df = 2, 22; $P = 0.03$) (Table 2). Flies spent ≈10-fold more time, on average, within 5 cm of GF-120 droplets than near water droplets. Although flies were observed feeding on droplets of all treatment types, the sample size observed was too small for comparison among treatments.

## Discussion

Unbaited and baited formulations of spinosad show promise for control of two of the most commercially important temperate fruit fly species, *R. pomonella* and *R. mendax.* Substantial levels of control were achieved for both *Rhagoletis* species when their respective host plants were treated with spinosad bait or SpinTor at recommended rates (see *Materials and Methods*). In apples, spinosad bait provided 67% reduction in fruit fly infestation in 2003, whereas in blueberries there were 85% (2002) and 98% (2003) reductions in fly infestations compared with controls. GF-120 fell short of outperforming the unbaited formulation of spinosad, but it did provide equivalent control by using only 0.5–1.5% as much active ingredient.

Although spinosad bait achieved a high degree of reduction in fruit infestation, it did not eliminate fruit infestation or significantly control adult *Rhagoletis* flies. For an insecticide-bait to be commercially viable for fruit fly control, it must provide extremely high fruit protection to allow growers to meet the stringent quality standards of the market. At least one infested blueberry or apple was collected in plots treated with GF-120 or SpinTor in 2002 and 2003. Complete suppression of fly infestation did not occur in experiments conducted with spinosad formulations on Mediterranean fruit fly (Peck and McQuate 2000, Vargas et al. 2002) or the melon fly, *Bactrocera cucurbitae* Coquillett, (Prokopy et al. 2003); yet, these products are used commercially for fly control. Similarly, SpinTor did not prevent larval infestation of apples in small (single-tree) plots with large apple maggot populations (Reissig 2003). In the current study, infestation of fruit in the relatively small test plots may have been due to immigration of flies from nearby untreated areas; therefore, future tests of SpinTor or spinosad bait should be conducted at a larger scale. Coverage of larger areas with these products may provide commercially acceptable control.

Observations of *R. mendax* in blueberry plantings revealed that similar numbers of flies visited blueberry plants treated with spinosad bait, bait, or water. In contrast, more flies were observed within proximity (5 cm) of spinosad bait and bait droplets than water droplets. Similarly, Mediterranean fruit flies exhibit attraction to GF-120 droplets on coffee plant leaves only when they are within several centimeters (Barry et al. 2003). In addition, *R. mendax* remained close to the droplet (within 5 cm) for significantly longer durations than near control water droplets. As defined by Kennedy (1978), an arrestant chemical is that which causes orthokinetic movement (the insect alters its rate of movement) or klinokinetic movement (the insect alters its rate of turning). Alternatively, an attractive chemical is that which causes nonrandom movement, orientational movement of the insect toward the stimulus. The results presented in Table 2 suggest that flies exhibit arrestment after close proximity with the bait component of GF-120, rather than attraction.

Prokopy et al. (2003) demonstrated that significantly fewer melon flies contacted GF-120 applied to sorghum, *Sorghum* sp. (nonhost), plants surrounding host cucumbers 1–4 d after application compared with freshly applied GF-120. In addition, GF-120 aged for 4 d under dry conditions retained one-half of its initial toxicity to melon flies despite the finding that a complete loss in attractiveness occurs after only 1 d under dry greenhouse conditions. In that study, stochastic (accidental) interactions between flies and the phagostimulants in GF-120 were the suggested mechanism by which ingestion of spinosad occurred. The observational findings reported here support the hypothesis that arrestment behavior, rather than attraction, is the underlying mechanism for the efficacy of GF-120.

The bait component of GF-120 contains only 1% (wt:vol) of the attractant ammonium acetate (Moreno and Mangan 2003). Field studies (Liburd et al. 1998, K.S.P., unpublished data) have shown that ammonium acetate in Polycon dispensers or incorporated into Pherocon AM traps dissipates after ≈2 wk in the field, resulting in an almost total loss of attractiveness. Rapid volatilization of ammonia from spinosad bait may explain the lack of attraction reported herein. Several studies indicate that increasing the amount of ammonium acetate or ammonium bicarbonate has a repellant effect on tropical fruit flies (Heath et al. 1995, Robacker and Moreno 1995). In contrast, a recent study showed that captures of *R. pomonella* increased in response to higher concentrations of ammonia (0–29.3%) released from ammonium hydroxide lures (Yee and Landolt 2004). Future studies should test the effects of increasing the percentage of ammonium acetate in spinosad bait on temperate fruit flies. If a higher rate of ammonium acetate enhances the duration of arrestment on spinosad bait, or triggers attraction of flies, it would be expected to increase the interaction of flies with the toxicant and the likelihood of preventing larval infestation.

Some technical challenges must be addressed to make the use of spinosad bait practical on a commercial scale. The current formulation must be applied frequently and requires reapplication after moderate-to-heavy rainfall (Prokopy et al. 2003). Modifications of the formulation to increase rain-fastness would greatly enhance its suitability for control of fruit flies in both temperate and tropical climates. Furthermore, the efficacy of spinosad bait may be improved by incorporation of host fruit volatiles, similar to the use of apple volatiles to enhance *R. pomonella* captures on spheres (Reynolds and Prokopy 1997, Prokopy et al. 2000, Stelinski and Liburd 2002). In addition, increasing the amount of ammonium acetate in the GF-120 formulation from 1% could potentially enhance its capacity to attract flies for a greater duration after application, and over a greater distance.

Pesticide-treated sphere traps that mimic host fruit have been the recent focus of bait-and-kill technology against *Rhagoletis* fruit flies, and these have provided effective control of apple and blueberry maggot flies (Duan and Prokopy 1995, Liburd et al. 1999, Hu et al. 2000, Stelinski and Liburd 2001, Stelinski et al. 2001). However, commercial use of attracticidal sphere traps has been limited due to the high rate of sphere deployment and cost of this approach (Stelinski et al. 2001). Baited insecticide formulations fit into the current system of pest control, which is based on the use of sprayable pesticides, whereas using pesticide-treated spheres for large-scale control requires a shift in convention. Sprayable formulations of spinosad and protein bait offer several other advantages, including ease of application, lower dosage of active ingredient, and reduced impact of insecticide load on the environment and nontarget insects (Vargas et al. 2001, 2002; Mazor et al. 2003). Due to their less restrictive preharvest intervals, compared with conventional insecticides, bait-and-kill formulations are promising for fruit protection closer to harvest when growers have fewer control options available.

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