Paraffin Wax Emulsion for Increased Rainfastness of Insecticidal Bait to Control *Rhagoletis pomonella* (Diptera: Tephritidae)

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ABSTRACT In regions with a humid summer climate, the use of water-soluble bait to control apple maggot is often limited by rainfall. We studied increasing the rainfastness of GF-120 fruit fly bait by adding paraffin wax emulsion. First, we verified that adding 10% wax to a mixture containing 16.7% GF-120 did not reduce the mortality of female apple maggot compared with GF-120 without wax. In addition, we determined that fly mortality caused by GF-120 plus wax subjected to simulated rain was similar to that caused by GF-120 without wax not subjected to rain. Other assays showed that higher fly mortality resulted from increasing the proportion of wax from 10 to 15%, and lower mortality resulted from decreasing GF-120 from 16.7 to 10 or 5%. The availability of spinosad on or near droplets of a mixture consisting of 5, 10, or 15% GF-120 and 15% wax was determined before and after the droplets were subjected to three 15-min periods of simulated rain. We found an initial steep decline in dislodgeable spinosad and smaller decreases after subsequent periods of rain. In a small-plot field trial, fruit infestation by apple maggot in plots treated with a mixture consisting of 16.7% GF-120 and 19.2% wax was less than in plots treated with 16.7% GF-120 without wax but not less than in control plots. Overall, we found that adding paraffin wax emulsion to GF-120 increased rainfastness in laboratory bioassays, and specifically that it retained the active ingredient spinosad. However, our field data suggest that optimal rainfastness requires the development of mixtures with >19.2% wax, which may preclude application using standard spray equipment.

KEY WORDS rainfastness, paraffin wax emulsion, GF-120, spinosad

Apple maggot, *Rhagoletis pomonella* (Walsh), is one of the most important pests of apples in eastern and midwestern North America (Prokopy et al. 2000, Myers et al. 2008). The apple maggot originally infested the fruit of several species of hawthorn, *Crataegus* spp., but shifted to apples after these were introduced into its range (Bush 1966). Presently, populations adapted to apple, *Malus domestica* Borkh., thrive in reservoirs such as abandoned apple orchards (Reissig 2003). Flies from these habitats disperse into apple orchards and constitute a direct threat to Michigan's apple crop, which is worth 130 million dollars and grown on 14,160 ha, the third largest growing area in the United States (USDA 2008). Apple maggot is a quarantine pest, and there is zero tolerance for fruit fly larvae in harvested fruit whether destined for processing or the fresh market. Although larvae are the damaging lifestage and the focus of grading standards, they are extremely difficult to control with insecticides because they live in the pulp of the fruit. Consequently, management of apple maggot requires that females be controlled before they lay eggs into the fruit (Howitt 1993, Wise et al. 2008). This is one of the main reasons that fruit growers apply neurotoxic insecticides throughout the period of adult activity. In recent years, the availability of effective organophosphates declined as a result of the implementation of the Food Quality Protection Act (EPA 1996). Consequently, growers are seeking new means for control of apple maggot.

The development and commercial availability of safer insecticides that act on flies mostly by ingestion (e.g., spinosad), has prompted renewed interest in the use of insecticidal baits for control of fruit-infesting *Rhagoletis* pests (Barry et al. 2005, Pelz et al. 2005, Yee and Chapman 2005). Research on bait sprays for control of *Rhagoletis* and other tephritid fruit flies has a long history. Severin et al. (1914) mentioned spraying bait for control of apple maggot and other species of *Rhagoletis.* Severin (1916) used sugar molasses with lead arsenate and copper acetoarsenite so flies would ingest the toxins and thereby increase their efficacy for control of apple maggot. A breakthrough in bait efficacy occurred when Steiner (1952) added protein hydrolysate to sugar for control of the oriental fruit fly *Bactrocera dorsalis* Hendel in Hawaii. Including protein hydrolysate in the bait mixture added a volatile attraction component to the phagostimulatory effect of sugar. The most used insecticide was malathion at the conventional ratios of one part insecticide to three or four parts bait (Roessler 1989). Concerns about the

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toxicity and environmental risks of malathion and greater availability of safer insecticides led to the development of baits that were more effective phagostimulants (Moreno and Mangan 2002). As a result of this research, there are now insecticidal baits such as GF-120 (Dow AgroSciences, Indianapolis, IN) that combine low rates of insecticide with bait specifically formulated to increase ingestion by flies. In addition, GF-120 is approved for organic management of fruit fly pests (OMRI 2008). However, the degree of protection against infestation by *Rhagoletis* fruit flies provided by currently available insecticidal baits still does not meet the stringent quality standards imposed by the market (Pelz et al. 2005) and can put growers at risk of crop rejection by processors.

Bait components such as sugar and protein hydrolysate are highly water soluble, and in areas with a humid summer climate, baits are not sufficiently rainfast for use at the standard 14-d spray interval preferred by most growers (L.T., unpublished data). To address this limitation of standard fruit fly baits, our research goal was to develop a bait mixture that could withstand rain events lasting up to 1 h or up to 25 mm of precipitation while maintaining the efficacy of bait not exposed to rain. We also wanted to develop a rainfast mixture that could be applied using standard spraying equipment. Based on our previous experience with wax matrix as the carrier material for insect pheromones, we chose paraffin wax emulsion to address the research goals. Emulsified paraffin wax has been successfully used as the carrier material for deploying sex pheromones for mating disruption of Lepidopteran pests (Atterholt et al. 1998, Delwiche et al. 1998, Atterholt et al. 1999, Stelinski et al. 2005, de Lame et al. 2007). Among the advantages of this method is the slow release of the pheromone over an extended period of time and the rainfastness (Atterholt et al. 1998, de Lame et al. 2007). Moreover, paraffin wax emulsion is nontoxic, may be approved under organic standards, is inexpensive, and can be manufactured relatively easily. A potential disadvantage of using paraffin wax emulsion is the presence of wax residue on the surface of harvested fruit. Bait must be sprayed during the flight period of apple maggot, which is coincident with fruit maturation and before harvest. Therefore, wax emulsion may be most suitable in orchards where fruit are destined for processing or are thoroughly cleaned before reaching the consumer.

In this study, we investigated the performance of an emulsified paraffin wax matrix in improving the rainfastness of GF-120 fruit fly bait. We conducted bioassays measuring mortality of apple maggot flies exposed to bait plus wax emulsion that had been previously subjected to simulated precipitation. We measured the amount of spinosad that is available on or around droplets of bait plus wax emulsion repeatedly subjected to simulated rain. Finally, we conducted a small-plot field trial with bait plus wax emulsion and determined fruit infestation at harvest.

Materials and Methods

Insects. Apple maggot pupae were obtained from infested apples collected in August 2006 in Fennville, MI. Pupae were stored at 4°C for at least 3 mo and brought to 21–23°C as needed. After emergence, males and females were kept together in 30 by 30 by 30-cm plastic cages (Bioquip, Rancho Dominguez, CA). Flies were kept in the same laboratory where experiments were conducted at 21–23°C. Food consisted of a 1:3 mixture of yeast protein hydrolysate and sugar. Food and water were provided separately. Food was removed from the cage 18–24 h before flies being assayed. Only female flies aged at least 5 d were used in these experiments to ensure they were reproductively mature.

Paraffin Wax Emulsion. The paraffin wax emulsion was prepared according to the protocol of de Lame (2003), modified for our purposes. Briefly, the formulation consisted of food-grade paraffin wax (Hobby Lobby, Oklahoma City, OK), Span 60 (sorbitan monostearate) as an emulsifier (Sigma-Aldrich, St. Louis, MO), and deionized water. Paraffin wax and water were heated separately to 65–70°C. When both had reached this temperature, Span 60 was incorporated into the wax, followed by the addition of the hot water. The resulting fluid was mixed for ~5 min in an industrial laboratory blender (Waring Commercial, Torrington, CT). The emulsion was gradually cooled to room temperature by placing the mixing bowl in cold water. Intermittent mixing during the cooling process was necessary to ensure that the final emulsion was smooth. The wax emulsion was stored in plastic containers and mixed with GF-120 as needed. For laboratory bioassays, we prepared 20% wax emulsion consisting of 20% (wt:wt) paraffin wax, 0.8% Span 60, and 79.2% deionized water. For field trials, we prepared 23% wax emulsion consisting of 23% (wt:wt) paraffin wax, 0.9% Span 60, and 76.1% deionized water.

From here on, we refer to the volume of wax in the final mixture, not wax emulsion, when describing the composition of mixtures of GF-120 and wax emulsion. Also, we refer to GF-120 mixed with paraffin wax emulsion as GF-120 + wax.

Optimal Composition of GF-120 + Wax. In our laboratory experiments, designed to study the rainfastness of mixtures containing different proportions of GF-120 and wax emulsion, we began by depositing 25-μl droplets of different mixtures on marked ornamental *Ficus* or apple leaves using a pipettor and allowing the droplets to dry for 24 h in a greenhouse. *Ficus* leaves were used initially because this study started in early spring when apple leaves were not available. Later, we used apple leaves on branches collected from an unsprayed apple orchard to better replicate conditions in the field. The *Ficus* plants or apple branches were placed in a rain simulator booth, at MSU's Trevor Nichols Research Complex, and exposed to rain. Briefly, the rain simulator booth consisted of a moving sprayer with interchangeable nozzles and a control valve for varying the water pressure.

Different rain patterns were created by using different nozzles. Sprayer speed and water pressure were controlled electronically. The sprayer used water purified by reverse-osmosis (Culligan, Rosemont, IL), kept in a storage tank at room temperature. Rainfall was measured as the average level of three rain gauges placed near the plant material. Marked leaves were allowed to dry before being brought to the laboratory for use in various assays.

Leaves containing bait deposits were placed at the bottom of 0.95-liter plastic containers on a wet cotton pad. The bioassays were conducted using five replicate containers per bait treatment, each replicate consisting of six female flies exposed to a treatment leaf. Blocks consisted of one replicate of each treatment kept together in the same tray. All replicates were initiated the same day, with flies from the same cohort. Food consisted of the same adult diet as described above that had been spread on 4-cm^2 strips of absorbent paper. Water was provided separately using a wet cotton pad. Flies were offered food on alternate days only. With this procedure, we maintained flies alive for the duration of the trials while increasing the probability that flies interacted with the bait. Food strips were placed in the container on the first day of the trial. Fly mortality was determined daily for 4 d. We analyzed mortality data recorded 3 d after the beginning of each trial because fly mortality in all treatments had stabilized by then.

A series of preliminary experiments had shown that adding paraffin wax emulsion to GF-120 protected the bait from the mechanical action of simulated rain. In the first experiment included in this section, our objectives were to establish (1) that adding wax to GF-120 did not decrease the efficacy of the mixture and (2) that adding wax increased the rainfastness of GF-120 subjected to simulated rain. We compared the mortality of flies that were exposed to treatments of GF-120, GF-120 subjected to simulated rain, GF-120 + wax, GF-120 + wax subjected to simulated rain, or an untreated control. GF-120 + wax consisted of 16.7% GF-120 and 10% wax, and it was prepared by mixing the appropriate volumes of GF-120, 20% paraffin wax emulsion and water. GF-120 was used diluted 1:5 with water to 16.7% because field data indicated that this dilution, the highest recommended for use in the field, was necessary for adequate plant coverage (L.T., unpublished data). The concentration of wax was chosen based on preliminary assays showing that it increased rainfastness while keeping the mixture fluid enough for spraying. Droplets deposited on *Ficus* leaves were subjected to 2.5 mm of rain for 5 min. In this assay, we used a TeeJet AT 1106U5 spray nozzle to generate rainfall droplets (TeeJet, Wheaton, IL). The sprayer speed was 5.8 m/s, and the pressure was 103.4 Pa.

In the second experiment in this section, we studied whether increasing the concentration of wax in the mixture could compensate for a relatively low concentration of GF-120. We compared the mortality of flies that were exposed to treatments of GF-120 + wax consisting of 5% GF-120 and 15% wax to that of flies exposed to 5% GF-120 and 10% wax, 10% GF-120 and 10% wax, 16.6% GF-120 and 10% wax, or an untreated control. All treatments were deposited on marked apple leaves and subjected to 30 mm of rain for 1 h, using a Teejet TTVP 1104 spray nozzle. The sprayer speed was 0.6 m/s, and the pressure was 68.9 Pa.

In the third experiment in this section, we determined whether using 15% wax in the mixture allowed using low concentrations of GF-120 without affecting overall efficacy. We compared the mortality of flies that were exposed to treatments of GF-120 + wax consisting of 5, 10, or 16.7% GF-120 or an untreated control. All treatments were deposited on apple leaves and subjected to 7 mm of rain for 1 h, using a Teejet TTVP 1104 nozzle. The sprayer speed was 2.9 m/s, and the pressure was 137.9 Pa.

Spinosad Dislodgeable Residue. The mode of action of spinosad, the active ingredient in GF-120, requires that the insects ingest the compound. Therefore, GF-120 + wax droplets have to offer bait containing spinosad in a form that is easily accessible for the flies to feed. Before GF-120 + wax droplets are subjected to rain, there is spinosad in GF-120 on the surface. Immediately after exposure to rain, only spinosad in the interior of the wax matrix remains. However, preliminary experiments had shown that droplets of GF-120 + wax were lethal to flies even after being subjected to repeated periods of simulated rain followed by gradual drying of the droplet. In addition, flies were observed feeding on the surface of droplets of GF-120 + wax and on deposits formed by bait that exudes from drying droplets. Flies never fed through the hardened wax matrix (L.T., unpublished data). Here, we determined how concentrations of 5, 10, or 15% GF-120, in a mixture containing 15% wax, affected the amount of dislodgeable spinosad before and after droplets were subjected to simulated rain. We defined dislodgeable spinosad as spinosad that is removed by gently washing the surface of the droplets with a small volume of water and considered it to be an estimator of the amount of spinosad that was available for flies to ingest.

We deposited five 25-μl droplets of the bait mixtures on 4.7-cm-diameter polyethylene lids using a pipettor. The droplets were allowed to dry for 24 h in a fume hood. The lids were attached to a stand at a 45° angle in a completely randomized block design with blocks consisting of three adjacent lids containing one of the treatments. The experimental design consisted of four blocks of three replicate lids each. The amount of dislodgeable spinosad was measured before exposure to simulated rainfall, and after each exposure to three 15-min periods with cumulative rainfall of 5, 13.5, and 26 mm. In this assay, we used a Teejet TTVP 1104 nozzle, the sprayer speed was 0.6 m/s, and the pressure was 68.9 Pa. We allowed droplets to slowly dry after each exposure to rain so that spinosad was released from the wax matrix. When the droplets were dry, the surface and a small area of the lid surrounding the droplet was gently washed using 1 ml of water and a pipettor with a 1,000-μl tip.

The liquid containing spinosad residue was collected in 1.5-ml glass vials and immediately brought to

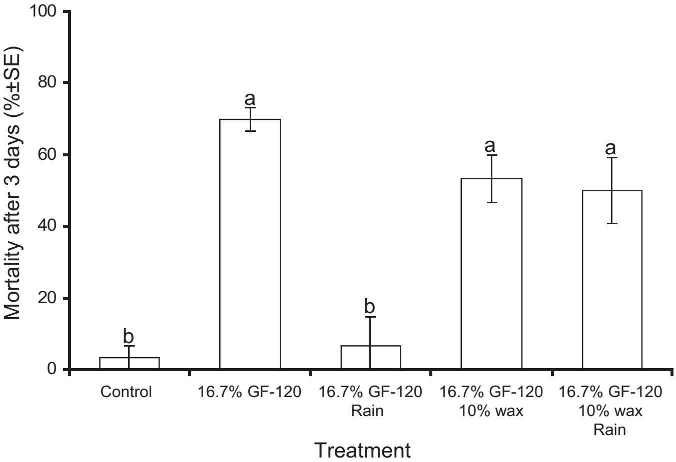

Fig. 1. Mortality of female apple maggot flies (mean ± SE) exposed for 3 d to leaves containing 25-μl bait droplets consisting of 16.7% GF-120, or 16.7% GF-120 + 10% wax, subjected or not subjected to 2.5 mm of simulated rain for 5 min, and an untreated control. Vertical bars with the same letters are not significantly different (LSD, $P > 0.05$).

MSU's Analytical Pesticide Laboratory, where the concentration of spinosad in the liquid was determined using standard methods (West and Turner 2000). We calculated the amount of dislodgeable spinosad per droplet because a few droplets in some of the lids became detached and were lost during the last period of exposure to simulated rain.

Small-Plot Field Trial. Visual inspection of GF-120 + wax deposits generated in preliminary field applications indicated that mechanical damage to the droplets from rainfall was more intense than when using simulated rain. Therefore, it was decided to use 19.2% of wax, the highest concentration of wax suitable for our standard spray equipment. We compared fruit infestation in apples treated with GF-120, GF-120 + wax, and an untreated control, in a small-plot field trial. GF-120 was diluted 1:5 with water to 16.7%, and GF-120 + wax contained 16.7% GF-120 and 19.2% wax. We used 30 ml of undiluted GF-120 per tree, the lowest rate recommended for spot treatments of GF-120. Each treated tree was sprayed with 180 ml of bait mixture, using an Ortho manual pressure sprayer (Scotts, Marysville, OH). The experiment was conducted using a randomized complete block design with four replicates consisting of 1-tree plots in the same row, in a large unsprayed orchard of the cultivar Idared with a history of apple maggot infestation, located in Fennville, MI. Each block was separated by at least 20 m. Plots were sprayed every 10 d, depending on weather conditions, starting on 6 July 2007. The five spray dates were 6 July, 20 July, 30 July, 9 August, and 17 August. On 27 August, 100 random apples were collected from each tree and brought to the laboratory. There, apples were placed on wire screens over moist sand so that apple maggot larvae exited the fruit and pupated in the sand. Six weeks later, the sand was sifted, and pupae were counted. We obtained weather records from a weather station located ≈500 m from the orchard. Apple maggot flight phenology was obtained from ammonium carbonate–baited Pherocon AM traps placed in another block by Trevor Nichols Research Station staff, ≈200 m from the trial site.

Data Analyses. Laboratory biossay mortality data expressed as a proportion were arcsine-transformed for homogeneity of variance and analyzed in a randomized complete block design with treatment as fixed, and block as random factors, using PROC MIXED and macro PDMIX800 of SAS (SAS Institute 2001). Differences among treatment were determined using the least significant difference (LSD) method with $\alpha = 0.05$. Dislodgeable spinosad residue data were analyzed using similar methods as above, except that the data were not transformed. The number of larvae that exited fruit collected from trees sprayed with different bait was log-transformed and analyzed using the same methods as above.

Results

Optimal Composition of GF-120 + Wax. Mortality of flies exposed to leaves containing GF-120, GF-120 + wax, or GF-120 + wax subjected to simulated rain was significantly higher than the mortality of flies exposed to control leaves or leaves containing GF-120 subjected to rain ($F = 24.2$; df = 4,16; $P < 0.001$; Fig. 1). There were no significant differences among the mortality of flies exposed to GF-120, GF-120 + wax, or GF-120 + wax subjected to rain. Likewise, there were no significant differences among the mortality of flies exposed to control leaves or leaves containing GF-120 subjected to rain.

The mortality of flies exposed to 5% GF-120 + 15% wax was significantly higher than the mortality of flies exposed to 5% GF-120 + 10% wax, 10% GF-120 + 10% wax, 16.6% GF-120 + 10% wax, or an untreated control

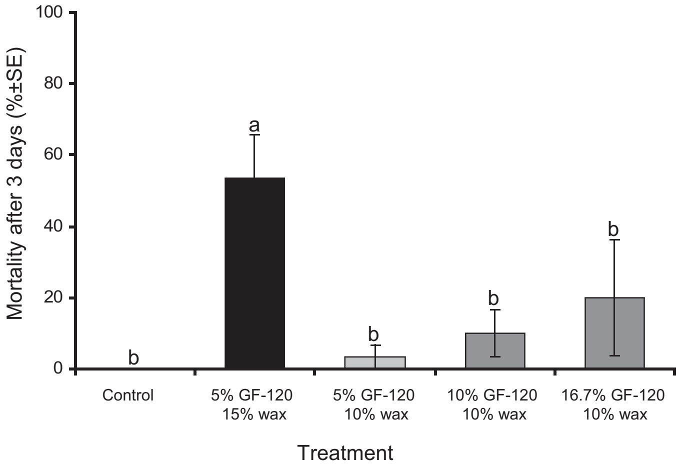

Fig. 2. Mortality of female apple maggot flies (mean ± SE) exposed for 3 d to leaves containing 25-μl bait droplets consisting of 5% GF-120 + 15% wax, 5% GF-120 + 10% wax, 10% GF-120 + 10% wax, 16.7% GF-120 + 10% wax, or an untreated control, all subjected to 30 mm of simulated rain for 1 h. Vertical bars with the same letters are not significantly different (LSD, $P > 0.05$).

($F = 6.0$; df = 4,16; $P = 0.004$), where all treatments were subjected to simulated rain (Fig. 2). There were no significant differences among the mortality of flies exposed to 5% GF-120 + 10% wax, 10% GF-120 + 10% wax, 16.6% GF-120 + 10% wax, or an untreated control.

The mortality of flies exposed to 16.7% GF-120 + 15% wax was significantly higher than the mortality of flies exposed to 5% GF-120 + 15% wax, 10% GF-120 + 15% wax, or an untreated control ($F = 15.0$; df = 3,12; $P < 0.001$), where all treatments were subjected to simulated rain (Fig. 3). There were no significant differences among the mortality of flies exposed to control, 5% GF-120 + 15% wax, or 10% GF-120 + 15% wax.

Spinosad Dislodgeable Residue. After a large initial decline in dislodgeable spinosad, we found that the release rate decreased, and there was spinosad available even after exposure to a total of 26 mm of rain during a 45-min period (Fig. 4). Before simulated rain, significantly more spinosad was present in treatments consisting of 10 and 15% GF-120 than in the treatment consisting of 5% GF-120 ($F = 35.2$; df = 2,16; $P < 0.001$). After 15 min under simulated rain, the amount of spinosad dropped by 88%. Now, significantly more spinosad was present in the treatment consisting of 15% GF-120 than in the treatment consisting of 5 or 10% GF-120 ($F = 12.0$; df = 2,16; $P = 0.008$). The amounts of dislodgeable spinosad found after the two subsequent 15-min exposures to rain declined further

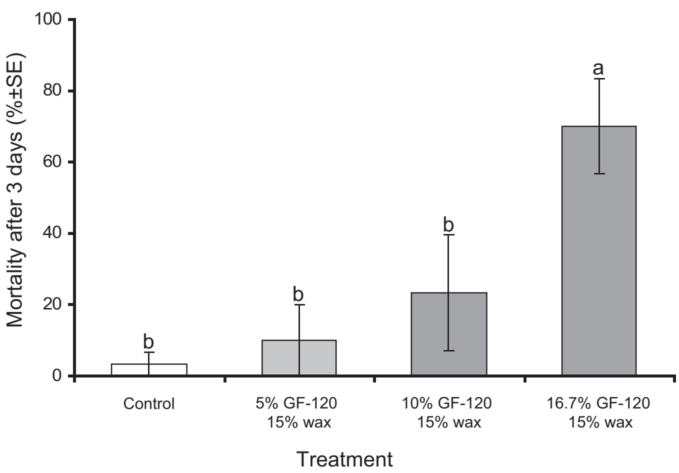

Fig. 3. Mortality of female apple maggot flies (mean ± SE) exposed for 3 d to leaves containing 25-μl bait droplets consisting of 5, 10, or 16.7% GF-120 + 15% wax, all subjected to 7 mm of simulated rain for 1 h. Vertical bars with the same letters are not significantly different (LSD, $P > 0.05$).

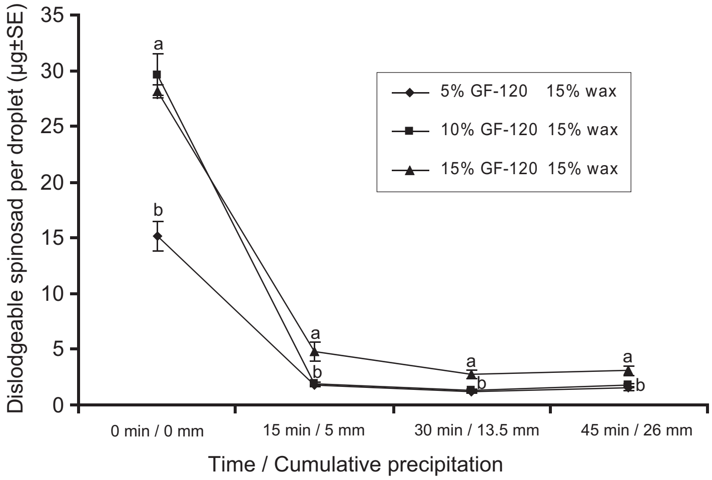

Fig. 4. Dislodgeable spinosad available on or near a 25-μl bait droplet (mean ± SE) before simulated rain and after the droplet was subjected to 5-mm precipitation for 15 min, 13.5-mm cumulative precipitation for 30 min, and 26-mm cumulative precipitation for 45 min. Averages within each time step with the same letters are not significantly different (LSD, $P > 0.05$).

but had similar relative amounts to that found after the first 15-min exposure to rain, with significantly more spinosad dislodgeable from droplets with 15% GF-120 (30 min: $F = 21.3$; df = 2,16; $P = 0.002$; 45 min: $F = 15.5$; df = 2,16; $P = 0.004$).

Small-Plot Field Trial. Infestation of fruit treated with GF-120 + wax was significantly less than fruit treated with GF-120 but not less than control fruit ($F = 5.3$; df = 2,6; $P = 0.047$; Table 1). Abundant rain occurred after the middle of the trial, with precipitation events above 10 mm recorded on eight of the days in this trial (Fig. 5). Throughout the trial, the daily maximum relative humidity was almost always near 100%. Capture of apple maggot flies in the vicinity of the orchard showed that this species was present during most of the trial and that captures peaked toward the end of the trial.

Discussion

This study showed that the addition of a paraffin wax matrix to insecticidal bait for control of apple maggot increased the rainfastness of the bait (Fig. 1). We verified that subjecting GF-120 to even a short, 5-min exposure to 2.5 mm of simulated rain was sufficient to decrease fly mortality to the level of the untreated control. The addition of a wax matrix to GF-120 had no negative effect on the efficacy of the mixture compared with GF-120 alone, when none of the treatments was exposed to simulated rain. In contrast, the efficacy of GF-120 + wax subjected to rain was significantly greater than the efficacy of GF-120 also subjected to rain and similar to that of GF-120 not subjected to rain. The other experiments helped elucidate how different concentrations of wax emulsion and GF-120 influence the rainfastness of the mixture (see below).

Increasing the concentration of paraffin wax emulsion from 10 to 15% increased the rainfastness of the mixture and caused fly mortality that was superior to that of mixtures containing less wax but the same or higher GF-120 concentration (Fig. 2). However, the use of 15% wax did not allow decreasing the concentration of GF-120 from 16.7 to 10 or 5% without causing a decrease in fly mortality (Fig. 3). Overall, rainfastness increased with higher wax concentration, but above ≈20%, the mixture became a paste that was impossible to spray with standard application equipment. We found in other trials that increasing the proportion of GF-120 in the mixture above 16.7% decreased rainfastness, likely because of excessive volume of soluble solids contained in the wax matrix (L.T., unpublished data). In this study, GF-120 was always used diluted at the lowest rate recommended for use in the field (1:5, or 16.7% in water) or at a lower concentration to preserve the rainfastness of the mixture. The concentration of the wax emulsion was always 19.2% or less for decreased viscosity.

By varying the concentration of GF-120 and measuring the amount of spinosad on and around droplets of GF-120 + wax subjected to simulated rain, our objective was to determine how the initial concentration of GF-120 influenced the amount of dislodgeable spinosad that can be ingested by the flies. We

Table 1. Average no. of apple maggot larvae that exited fruit collected from apple trees treated with 16.7% GF-120, 16.7% GF-120 + 19.2% wax emulsion, or an unsprayed control

Treatment	Average no. larvae per fruit ± SE
Control	1.8 ± 0.2ab
16.7% GF-120	2.1 ± 0.2a
16.7% GF-120 + 19.2% wax	1.1 ± 0.0b

Treatment means followed by the same letter are not significantly different (LSD, $P > 0.05$).

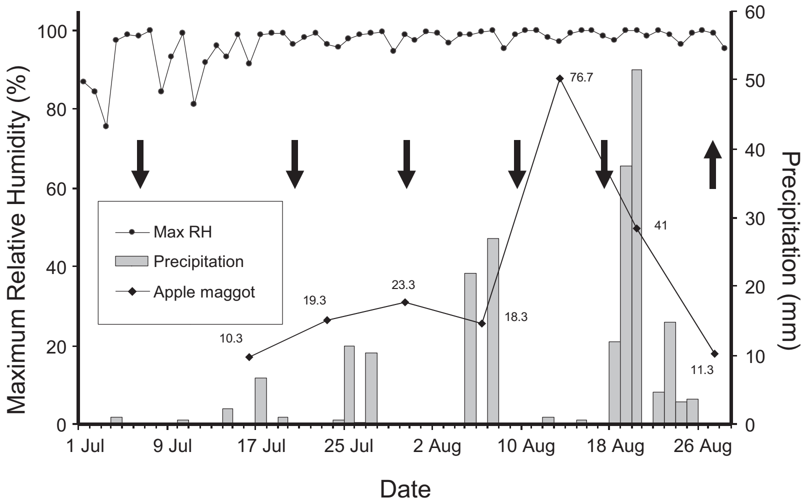

Fig. 5. Maximum daily relative humidity (%), daily precipitation (mm), and apple maggot capture in the vicinity of the field trial site, in Fennville, MI. Arrows pointing down indicate the dates bait sprays were applied, the arrow pointing up indicates the date of fruit collection.

found that dislodgeable spinosad was present on the droplets of GF-120 + wax even after the droplets were subjected to three 15-min periods of simulated rain. With all bait concentrations, spinosad release occurred in a pattern similar to the release of pheromone from other wax matrices (de Lame et al. 2007), with a large initial decline in dislodgeable spinosad and a sharp decrease in the release rate after subsequent periods of rain. However, spinosad becomes available for flies to ingest while the droplet dries after subjected to rain, whereas the release of pheromones through volatilization is continuous. The relationship between the amount of GF-120 in the mixture and the amount of dislodgeable spinosad changed before and after simulated rain, suggesting that different concentrations of GF-120 interacted with the wax matrix to create distinct patterns of release. This experiment also suggests that, in the third bioassay (Fig. 3), a higher amount of dislodgeable spinosad found on or around the droplet contributed to higher fly mortality in the treatment consisting of 16.7% GF-120 and 15% wax.

Our ultimate objective with the field trial was to compare the effectiveness of GF-120 and GF-120 + wax in protecting fruit from infestation. Our results showed that the performance of GF-120 + wax was significantly better than GF-120, even though the level of infestation was relatively high and not significantly different from the control (Table 1). Reproductively mature flies must find and ingest the bait before egg-laying for it to be effective, which may have been hard to accomplish using single tree plots because there was no inner area that would be protected by bait intercepting immigrating flies. Moreover, a 10-d spray schedule may have been too long, considering the relatively short activity of spinosad in the field (Liu et al. 1999, McLeod et al. 2002, Van Steenwyk et al. 2003, but see Yee and Alston 2006). Several other reasons may have contributed to the relatively poor performance of GF-120 + wax under field conditions. High fruit infestation may have resulted from the coincident occurrence of peak apple maggot flight and rain events far exceeding the rainfastness of GF-120 + wax. In addition, the droplets that resulted when GF-120 + wax was sprayed tended to spread much more than when the mixture was deposited on a leaf with a pipettor. The large droplet surface area may have contributed to loss of GF-120 from the matrix not just from rain but also from bait hygroscopicity. GF-120 was frequently observed leaching out of droplets that had been dry the previous day (L.T., unpublished data). This may have been caused by the high relative humidity in the morning almost every day during the trial (Fig. 5). The reliquefied bait eventually run off the leaves. Despite the relatively poor performance of GF-120 + wax, this trial helped elucidate the importance of droplet characteristics and relative humidity on the performance of the mixture.

Overall, we consider that adding an emulsified wax matrix to fruit fly bait has potential for increasing the rainfastness of bait. In humid climates where summer storms can cause intense precipitation events, the used of wax matrix will improve retention of the water-soluble bait within droplets but will not totally prevent its loss. Possible avenues to extend the performance of baits under humid climates may include developing application equipment that allows the use of wax concentrations above 19.2% in the mixture or searching for fly feeding stimulants that are not water soluble.

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