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FEEDING AND SURVIVORSHIP OF BLUEBERRY MAGGOT FLIES (DIPTERA: TEPHRITIDAE) ON PROTEIN BAITS INCORPORATED WITH INSECTICIDES

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ABSTRACT

Laboratory feeding trials evaluated fly survivorship on six insecticides (acetamiprid, clothianidin, deltamethrin, fipronil, imidacloprid, and spinosad) incorporated at 4, 40, and 400 ppm in protein baits. Higher concentrations of insecticides resulted in increased fly mortality. At all concentrations of insecticides in baits, except those on deltamethrin, there was a significantly higher mortality 4 d after the initial feeding, compared with flies that fed on a control bait. The presence of clothianidin or imidacloprid in baits led to significantly less feeding compared with a control bait without insecticide. There were no feeding deterrent effects of bait containing either fipronil or spinosad compared with a control bait without insecticide. Exposure of flies to fresh bait containing 40 ppm of acetamiprid, clothianidin, or imidacloprid, resulted in significantly more flies becoming knocked down than the control. Baits containing 40 ppm of fipronil or spinosad resulted in higher levels of fly mortality than baits containing either neonicotinoids (acetamiprid, clothianidin, or imidacloprid) or no insecticide for trials with fresh and 1-d-old bait with unlimited exposure. At the rates tested baits containing deltamethrin resulted in no fly knockdown and always had the lowest mortality of any insecticide treatment. The tradeoffs between insecticides capable of knockdown and mortality are discussed as they relate to management of *R. mendax*.

Key Words: *Rhagoletis mendax*, acetamiprid, clothianidin, fipronil, imidacloprid, spinosad

RESUMEN

La sobrevivencia de la mosca, *Rhagoletis mendax*, contra seis insecticidas (acetamiprid, clothianidin, deltamethrin, fipronil, imidacloprid, y spinosad) incorporados a 4, 40, y 400 ppm en cebos de proteina fue evaluada en pruebas de alimentación en el laboratorio. Las concentraciones mas altas de insecticidas resultaron en un aumento de la mortalidad de las moscas. En todas las concentraciones de los insecticidas en cebo, menos aquellas tratadas con deltamethrin, hubo una mortalidad significativamente mas alta 4 d después de la alimentación inicial, comparada con las moscas que se alimentaron sobre el cebo de control. La presencia de clothianidin o imidacloprid en el cebo resulto en una alimentación significativamente menor comparada con el cebo de control sin insecticida. No hubo ningún efecto detrimental en la alimentación del cebo que tenia fipronil o spinosad comparado con el cebo de control sin insecticida. La exposición de las moscas al cebo fresco con 40 ppm de acetamiprid, clothianidin o imidacloprid, resulto en significativamente mas moscas derribadas que en el control. Los cebos con 40 ppm de fipronil o spinosad resultaron en un nivel mas alto de la mortalidad de moscas que en los cebos con neonicotinoids (acetamiprid, clothianidin, o imidacloprid) o sin insecticida para las pruebas con cebo fresco y de cebo de un dia con exposición sin limite. A las tasas de insecticidas probadas, ningún mosca fue afectada en la prueba de cebo con deltamethrin y siempre tenian la menor mortalidad que cualquier otro tratamiento de insecticidas. Se commentan sobre los factores de los insecticidas con la capacidad para un efecto de noqueo de las moscas versus un insecticida que mata las moscas en relación al manejo de *R. mendax*.

The blueberry maggot fly, *Rhagoletis mendax* Curran, is a serious pest of lowbush and highbush blueberries, *Vaccinium angustifolium* Aiton and *V. corymbosum* L., respectively, in the northeastern United States and Atlantic Provinces of Canada. In areas not infested with *R. mendax* there is zero-tolerance for maggot presence. As a result, growers exporting fruit to non-infested areas of

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Canada must participate in a Blueberry Certification Program (Canadian Food Inspection Agency 1999).

This certification program mandates following either a calendar-based or an integrated pest management (IPM) spray program. A calendar-based approach requires growers to start spraying insecticides within 10 d of the first detection of an adult fly in the area, and continue spraying at 7- to 10-d intervals until the end of harvest. An IPM-spray program requires growers to monitor the presence of adults with traps baited with ammonium acetate. A recommended insecticide should be applied within 5 d of the date of capture of a single fly in any one of the monitoring traps, followed by a second spray 7-10 d later. This spray sequence should be repeated for each subsequent fly detection until the end of harvest. Many blueberry growers use one of these spray regimens, but there are several alternative strategies that have been investigated.

Rhagoletis flies can be controlled and managed by a variety of insecticides and application methods. Broad-spectrum insecticides, such as organophosphates and carbamates, have been applied in ultra low volume sprays, where contact and feeding toxicity of small droplets can cause fly mortality (Mohammad & Aliniazee 1989; Hu et al. 2000). The enactment of the Food Quality Protection Act (FQPA) (1996) has placed severe restrictions on the use of these broad-spectrum insecticides, and future management of *Rhagoletis* flies will involve the use of insecticides that are not impacted by FQPA reassessment. Many of these new compounds have little or no contact toxicity; therefore, they are often incorporated into a bait station or bait spray, in which mortality results after flies ingest significant quantities of insecticide.

Painted spheres baited with ammonia compounds are highly attractive to tephritids and have been developed as bait stations. Spheres were first coated with a sticky material to trap flies (Prokopy 1975), but the need for decreased deployment and handling time necessitated finding an insecticide replacement (Duan & Prokopy 1995b). Studies evaluating the effects of insecticides, which were incorporated into the paint and sugar matrix that coated the surface of spheres, have been performed for *Anastrepha ludens* (Loew) (Prokopy et al. 2000b); *Ceratitis capitata* (Wiedemann) (Hu et al. 1998); and *R. mendax* and *R. pomonella* (Walsh) (Duan & Prokopy 1995b; Liburd et al. 1999; Ayyappath et al. 2000; Stelinski et al. 2001). Comparisons between baited spheres and azinphos-methyl sprays in a commercial apple orchard showed similar reductions in populations of *R. pomonella* (Prokopy et al. 2000a). However, in commercial blueberry fields insecticidal spheres are not currently used because of the deployment density, lack of attractive selective lure, associated costs of products (i.e., spheres and residue extending agents), and labor requirements (i.e., monitoring and applying insecticides to spheres) (Barry et al. 2004).

Another alternative to broad-spectrum sprays are protein bait sprays which contain ammonia-based attractants, a feeding stimulant such as sucrose, and an insecticide. Protein bait sprays have been used to control outbreaks of *Anastrepha ludens*, *Bactrocera dorsalis* (Hendel), and *Ceratitis capitata* since the 1950s in the United States (Steiner 1952; Moreno & Mangan 2003). However, development and evaluation of protein bait sprays on *R. mendax* has begun only recently. Protein and ammonia-based attractants have been evaluated on *Anastrepha* spp. (Moreno & Mangan 2003), *B. cucurbitae* (Coquillett) (Fabre et al. 2003), *B. dorsalis* (Cornelius et al. 2000), *R. cerasi* (L.) (Katsoyannos et al. 2000), and *R. pomonella* (Duan & Prokopy 1992). Different concentrations of sugar feeding stimulants have been tested on *A. suspensa* (Loew) (Sharp & Chambers 1984), *R. pomonella* (Duan & Prokopy 1993), and *R. mendax* (Barry & Polavarapu 2004).

Dowell (1994) outlined alternatives to a malathion bait spray, which had been the preferred method in eradicating incipient infestations of *C. captitata*. Further development of a replacement has led to evaluation of insecticides classified as reduced risk. One compound that has already been incorporated into a bait spray for tropical and sub-tropical tephritid pests is spinosad, which was developed from the bacterium *Saccharopolyspora spinosa* Mertx and Yao. Feeding on baits containing spinosad has resulted in high mortality for *A. ludens* (Prokopy et al. 2000b), *A. suspensa* (King & Hennessey 1996), *B. cucurbitae* (Prokopy et al. 2003), and *C. capitata* (Peck & McQuate 2000; Vargas et al. 2001; Barry et al. 2003). Trials assessing toxicity have occurred for several of the non-organophosphate and non-carbamate compounds, such as deltamethrin, imidacloprid, and spinosad, on *R. pomonella* (Duan & Prokopy 1995a; Hu et al. 2000; Bostanian & Racette 2001; Reissig 2003) and acetamiprid, deltamethrin, fipronil, and imidacloprid on *R. mendax* (Barry et al. 2004). The reduced risk insecticide clothianidin, a neonicotinoid, has not been evaluated on any tephritid species.

Our goal was to identify the most effective concentrations of insecticides present in bait that resulted in knockdown, mortality, and had the least feeding deterrence on *R. mendax*.

Materials and Methods

Insects

Infested blueberries were collected near Chatsworth, NJ, in the summer of 2002 and 2003. The rearing procedures of Ayyappath et al. (2000)

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were used to obtain adult *R. mendax*. Briefly, infested berries were placed over moist sand for larvae to drop and pupate. Puparia were sifted from sand three-five weeks later and kept in a screenhouse. Puparia were transferred to an incubator on 1 November 2002 and 2 November 2003, at 6°C with a photoperiod of 12:12 (L:D) to complete diapause. On 27 March 2003 and 30 March 2004 puparia were placed at 8°C. Periodically groups of puparia were transferred from 8 to 15°C for approximately 8 d and then transferred to an incubator at 25°C with a photoperiod of 16:8 (L:D) until adult emergence, which occurred 25-45 d later. Adult flies were kept at 22°C and were provided a diet of sucrose and water (i.e., protein-starved). Flies used in assays were 7-13 d-old and allowed to acclimatize to experimental conditions in the laboratory for several hours before trials commenced.

Feeding Assay—Feeding for 10 s

In the laboratory (21-23°C), a no-choice feeding test was used to evaluate survivorship of *R. mendax* on a control bait with baits containing three concentrations (4, 40, and 400 ppm or 0.0004, 0.004, and 0.04% [AI], respectively) of six insecticides: acetamiprid (technical, 30% [AI]; Cerexagri, King of Prussia, PA), clothianidin (technical, 49.17% [AI]; Arvesta, San Francisco, CA); deltamethrin and imidacloprid (technical 99.1, and 98.9% [AI], respectively; Bayer, Kansas City, MO); fipronil (technical 88% [AI]; Aventris Crop Science, Research Triangle Park, NC); and spinosad (technical, 90.4% [AI]; Dow AgroSciences, Indianapolis, IN). Solutions of each insecticide concentration were prepared by weighing the appropriate amount of technical and then adding it to the corresponding 1:3-mixture of SolBait (prepared as a 2× concentrate, USDA-ARS, Weslaco, TX) and water. (A 1:3-mixture corresponds to a 1:4 mixture of GF-120 Fruit Fly Bait [Dow AgroSciences] to water.) After preparing the highest concentration, serial dilutions with a 1:3-mixture of SolBait were used to obtain mixtures with lower concentrations of insecticides. The control was a 1:3-mixture of SolBait to water containing no insecticide.

One 10-µl droplet of bait was placed on a white plastic lid (5.5 cm in diameter) located on top of a plastic cylinder (4 cm in diameter, 4 cm in height) in the center of a Plexiglas cage (30 cm × 30 cm × 30 cm). A fly was transferred to this lid and placed next to the droplet. After feeding on a droplet for 10 seconds, the fly was removed from the lid and placed inside a plastic cylinder (5 cm in diameter, 8.5 cm in height) containing water and sucrose. Flies that fed less than 10 seconds were discarded, unless it was determined that after the initial feeding a fly became incapable of feeding as a result of the insecticide (i.e., knockdown). A total of 30 flies were evaluated with the control and for each of three concentrations of insecticide (except deltamethrin which was not evaluated at 4 ppm).

Flies were assessed for knockdown (i.e., immobile or incapable of walking) 1 h after the 10-s feeding. The number of dead, active, and incapacitated flies was recorded after 1, 2, 3 and 4 d. Flies were characterized as dead if there was no presence of visible body movement (i.e., no leg twitch), active if able to walk, and incapacitated if incapable of walking (Hu et al. 2000; Reissig 2003). The number of living flies is represented by the sum of active and incapacitated flies.

Feeding Assay—Feeding for 5 min

In the laboratory (21-23°C), a no-choice test was used to evaluate feeding propensity of female *R. mendax* on protein bait containing 40 ppm of insecticide. Treatments were prepared by the methods described in the 10-s assay and included a control bait (without insecticide), clothianidin, fipronil, imidacloprid, and spinosad. One 10-µl droplet of a treatment was placed on a silk ficus leaf (Michaels, Irving, TX) that was placed on top of a plastic cylinder (4 cm in diameter, 4 cm in height) in the center of a Plexiglas cage (30 × 30 × 30 cm). Silk leaves were preferred to blueberry leaves because of the presence of chemical cues in the latter. One fly was transferred to the leaf within 1 cm of the droplet. Each feeding trial ended after 5 min if a fly was still present on a leaf or when a fly left a leaf after 5 s. (Flies that left a leaf in less than 5 s were not counted because they were believed to be in an agitated state.) In addition, all flies had to feed a minimum of 1 s on the droplet.

The amount of time that a fly spent feeding on a droplet was recorded. Flies were assessed for knockdown 1 h after feeding. Mortality was recorded 1 and 4 d after feeding. Each fly was tested only once. A total of 28 replicates were completed for fly feeding and 20 replicates were completed for knockdown and mortality. One replicate was completed after a female fly had been tested on four protein baits incorporated with different insecticides and the control bait.

Exposure—4 h

Survivorship and knockdown of flies was assessed to blueberry bushes treated with insecticidal baits. A control bait and four baits containing 40 ppm insecticide of clothianidin, fipronil, imidacloprid, and spinosad, were prepared by the methods described in the 10-s Feeding Assay. Bait was applied with a handheld sprayer (30 Gunjet; Spraying Systems Co., Wheaton, IL) to deliver three 1-ml squirts at 30 psi to each of four three-yr-old blueberry bushes. This rate is equivalent to 9 liters/ha (0.95 gallons/acre). Three branches

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(10-15 cm in length) were removed from each bush and placed inside a 250-ml Erlenmeyer flask in a Plexiglas cage (30 × 30 × 30 cm) that contained 20 flies (10 male, 10 female). The flask and branches were removed after 4 h. Flies were assessed for knockdown 3 h after introduction of treated branches and for mortality after 24 and 48 h. A total of 4 replicates were completed.

Unlimited Access—Fresh and 1-d-old bait

A no-choice assay evaluated fly mortality to bait containing the following insecticides: acetamiprid, clothianidin, fipronil, imidacloprid, and spinosad. Bait was prepared by adding enough technical insecticide to obtain 40 ppm [AI] in a mixture with GF-120 Fruit Fly Bait blank that did not contain spinosad (Dow AgroSciences). The control contained bait without the addition of insecticide. For each treatment, one 10-µl droplet of bait was applied to 60 highbush blueberry leaves. Half of these leaves were removed within 10 min of application for use in fresh assays and the other half remained on bushes for 24 h before being collected. Three treated leaves of the same bait were placed inside each of ten 1-liter plastic containers with a screened lid, which contained a moist cotton ball. Five flies were then placed in each container, which constituted a replicate. Flies were assessed for knockdown after 1 h and mortality 24 and 48 h after the start of exposure. Ten replicates were completed for fresh and 1-d-old bait. This experiment occurred in the laboratory where temperature was 21-23°C.

Statistical Analyses

Knockdown and survivorship data from the 10-s Feeding Assay are presented in tabular and graphical form, respectively. For this feeding assay, comparisons also were made between the control and each treatment with multiple chi-square tests after Bonferroni correction for the number of flies living versus dead after 4 d. In the 5-min assay feeding, duration was log transformed and analyzed by Fisher's least significant different (LSD) tests ($P = 0.05$). Knockdown and mortality were analyzed by multiple chi-square tests after Bonferroni correction. Prior to analysis of variance (ANOVA), mortality and knockdown were arcsine-square root transformed in both the 4-h exposure assay and the unlimited access assays (SAS Institute 1999). Means were separated by Fisher's LSD tests ($P = 0.05$).

RESULTS

Feeding Assay—10 s

Insecticide type and concentration resulted in different survivorship of living (active + incapacitated) and active flies (Figs. 1 and 2, respectively). After 4 d, 97% of flies fed the control bait were living, which was significantly higher than all treatments except 4 ppm of acetamiprid, clothianidin, and imidacloprid, and 40 and 400 ppm of deltamethrin (χ^2, with Bonferroni correction, $P = 0.05$). Greater than 90% of flies were living 4 d after feeding on bait containing 4 ppm of acetamiprid, clothianidin, and imidacloprid (Fig. 1A-C); whereas less than 10% were living after feeding on baits with the same concentration of fipronil and spinosad (Fig. 1E, F).

Four days after feeding on bait containing 400 ppm of insecticide, there were 13, 43, and 87% flies categorized as living for treatments of clothianidin, acetamiprid, and deltamethrin, respectively (Fig. 1A, B, D). At 400 ppm of fipronil, spinosad, and imidacloprid in baits it took 1, 3, and 4 d after treatment to reach 0% survivorship, respectively (Fig. 1C, E, F). Large decreases in survivorship occurred between 1 and 4 d for all concentrations of spinosad and 4 ppm fipronil.

Comparison of survivorship curves of living (active + incapacitated) with active flies appeared similar for treatments of clothianidin, deltamethrin, and fipronil (compare Fig 1B with 2B, 1D with 2D, 1E with 2E, respectively), but differed for the other three insecticides. The number of active flies increased from 1 to 4 d after feeding on bait containing 400 ppm acetamiprid, which indicated that some flies which had been incapacitated were now active (Fig. 2A). The percent of living flies compared with active flies was 40 and 3%, respectively, 1 d after feeding on bait containing 400 ppm imidacloprid, indicating that most (>90%) living flies were incapacitated (Fig. 1C and 2C, respectively). A large proportion of flies that fed on bait containing 40 and 400 ppm of spinosad were incapacitated, resulting in significantly fewer active than living flies 1-2 d after feeding (compare Fig. 1F with 2F).

More than 80% of flies were knocked down after 1 h on baits containing 400 ppm of acetamiprid, clothianidin, and imidacloprid, with 30% knocked down for fipronil and spinosad (Table 1). Flies exposed to treatments of deltamethrin and control bait were not affected. Compared with the control bait there were significant higher knockdown effects after 1 h for baits containing 40 ppm of acetamiprid (20%), clothianidin (80%), and imidacloprid (63%).

Feeding Assay—5 min

Protein baits containing insecticide had a significant effect on feeding duration ($F = 65.79$; $df = 4, 135$; $P < 0.0001$; Fig. 3). Compared with the control bait flies fed significantly less on baits containing imidacloprid and clothianidin, and fly feeding was not significantly different for baits containing fipronil and spinosad. Bait containing

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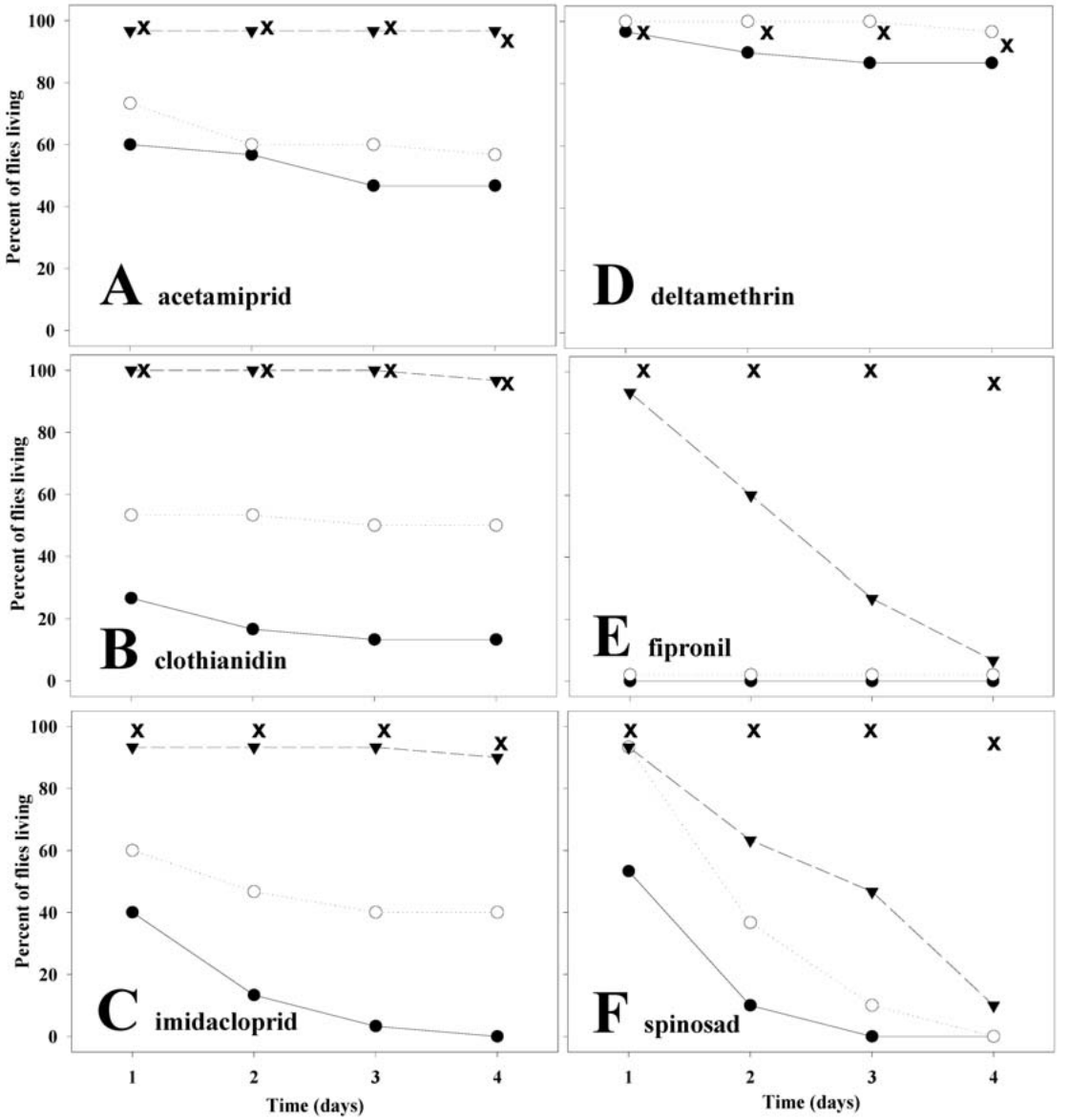


Fig. 1. Percent of flies that were living (active + incapacitated flies) after feeding for 10 s on a droplet of a given insecticide concentration. A) Acetamiprid, B) Clothianidin, C) Imidacloprid, D) Deltamethrin, E) Fipronil, F) Spinosad; (▼ = 4 ppm, ○ = 40 ppm, ● = 400 ppm insecticide; X = control bait without insecticide) (Deltamethrin was not evaluated at 4 ppm.)

imidacloprid was the only treatment that resulted in knockdown after 1 h that was significantly higher than the control (χ^2 with Bonferroni correction, $P = 0.05$; Table 2). Flies that fed on fipronil were dead after one day and flies that fed on spinosad were all dead after four days; and both results were significantly higher than the fly mortality in the control (χ^2 with Bonferroni correction, $P = 0.05$; Table 2).

Exposure—4 h

All insecticide treatments resulted in significantly higher knockdown than the control except spinosad after 3 h ($F = 4.47$; $df = 4, 15$; $P = 0.014$; Table 3). After 24 h, treatments had a significant effect on fly mortality ($F = 3.58$; $df = 4, 15$; $P = 0.031$; Table 3), with all insecticide treatments resulting in significantly higher mortality than the

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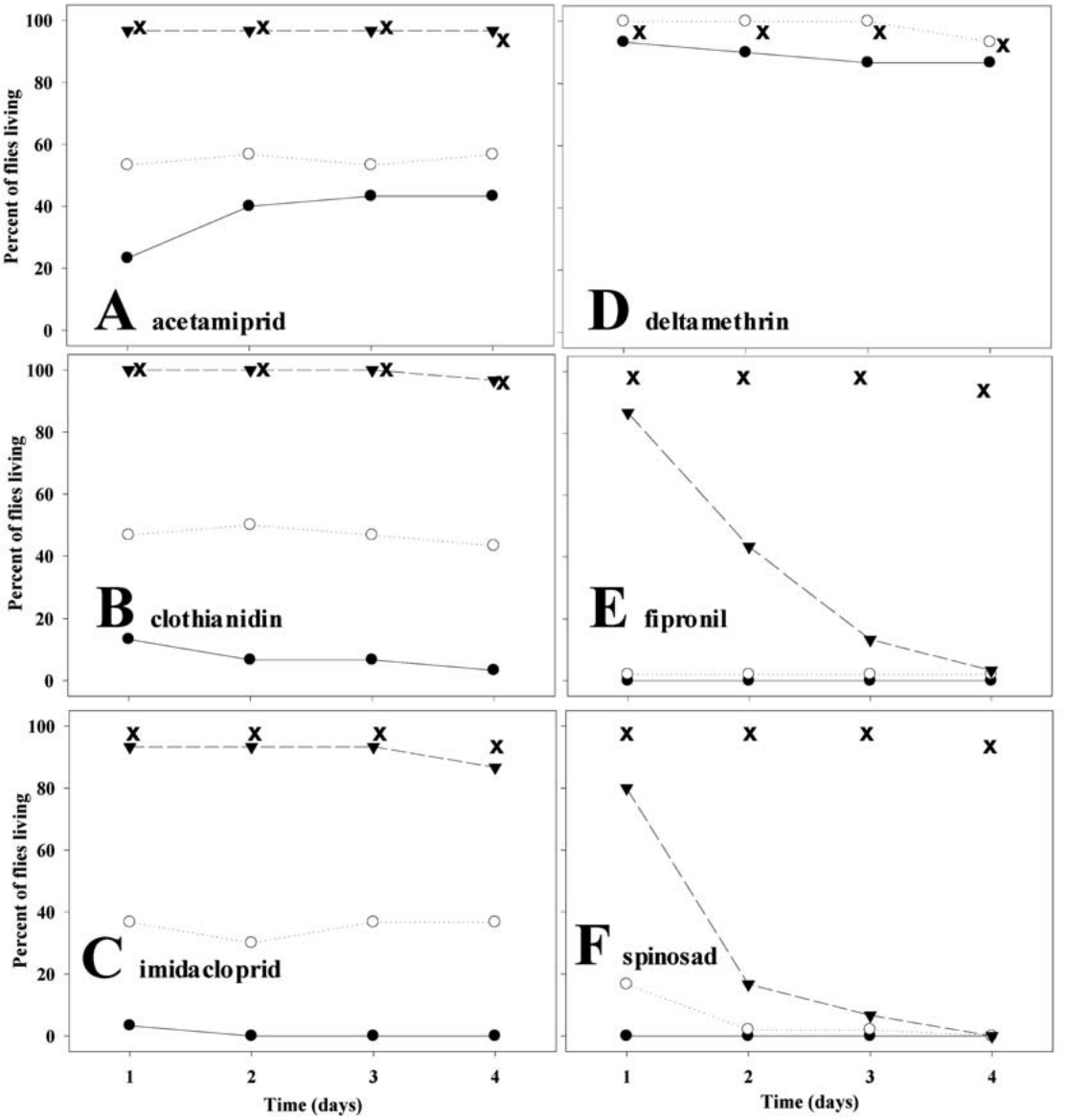


Fig. 2. Percent of flies that were active (with incapacitated flies excluded) after feeding for 10 s on a droplet of a given insecticide concentration. A) Acetamiprid, B) Clothianidin, C) Imidacloprid, D) Deltamethrin, E) Fipronil, F) Spinosad; (▼ = 4 ppm, ○ = 40 ppm, ● = 400 ppm insecticide; X = control bait without insecticide) (Deltamethrin was not evaluated at 4 ppm.)

control except imidacloprid. After 48 h, there were no differences among the treatments including the control (F = 1.12; df = 4, 15; P = 0.385; Table 3).

Survivorship Unlimited Access—Fresh bait

Feeding on fresh bait containing insecticide resulted in significant fly knockdown after 1 h (F = 7.45; df = 5, 54; $P < 0.0001$; Table 4). Significantly more flies were knocked down on treatments of bait containing acetamiprid, clothianidin, and imidacloprid compared with the control or treatments containing fipronil and spinosad. After 24 and 48 h, treatments had a significant effect on fly mortality (F = 14.86; df = 5, 54; $P < 0.0001$; and F = 15.65; df = 5, 54; $P < 0.0001$, respectively). After 24 h, fly mortality was significantly higher on fipronil and spinosad baits compared with the other three insecticide treatments, all of which

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TABLE 1. KNOCKDOWN OF FLIES 1 H AFTER FEEDING FOR 10 S ON INSECTICIDAL BAIT.

Treatment	Fly knockdown (%)		
	400 ppm	40 ppm	4 ppm
Acetamiprid	83 a	20 b	0
Clothianidin	96 a	79 a	0
Deltamethrin	0 c	0 b	—
Fipronil	30 b	0 b	0
Imidacloprid	100 a	63 a	3
Spinosad	30 b	0 b	0
Control	0 c	0 b	0
			NS

Values in the same column having the same letter are not significantly different (multiple chi-square tests after Bonferroni corrections; $P = 0.05$).
$n = 600$ flies.

were significantly higher than the control. These relative treatment relationships were the same after 48 h.

Survivorship Unlimited Access—1-d old bait

Treatments of 1-d old bait containing insecticides had a significant effect on fly knockdown ($F = 8.49$; $df = 5, 54$; $P < 0.0001$; Table 4). The highest numbers of flies were knocked down on acetamiprid, followed by imidacloprid, with both significantly higher than the control. The other three insecticides were not different from the control in fly knockdown. After 24 and 48 h, treatments had a significant effect on fly mortality ($F = 8.88$; $df = 5, 54$; $P < 0.0001$; and $F = 9.9$; $df = 5, 54$; $P < 0.0001$, respectively). After 24 h, fly mortality was

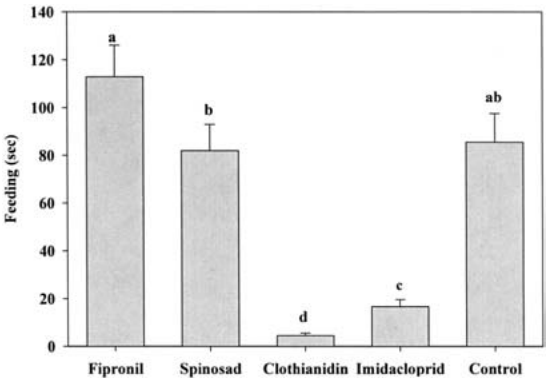


Fig. 3. Duration of fly feeding (mean ± SE) on a bait containing 40 ppm insecticide. Flies were allowed to feed a maximum of 5 min on a 10-µl droplet. The control was bait without insecticide. Vertical bars with the same letter are not significantly different. (Fisher's LSD test with log transformed data). ($F = 65.79$; $df = 4, 135$; $P < 0.0001$).

TABLE 2. FLY MORTALITY AND KNOCKDOWN AFTER 5 MIN EXPOSURE TO BAIT CONTAINING 40 PPM INSECTICIDE.

Treatment	Knockdown (%)	Mortality (%)	
	1 h	1d	4d
Clothianidin	55 ab	15 ab	25 ab
Fipronil	5 ab	100 a	100 a
Imidacloprid	80 a	20 ab	50 ab
Spinosad	5 ab	40 ab	100 a
Control	0 b	0 b	5 b

Values in the same column having the same letter are not significantly different (multiple chi-square tests after Bonferroni corrections; $P < 0.05$).
$n = 100$ flies.

significantly higher on fipronil and spinosad baits compared with the control and the other insecticide baits. After 48 h, baits containing fipronil and spinosad resulted in significantly higher mortality than baits containing either acetamiprid or clothianidin, with the latter two baits resulting in significantly higher mortality than either the control bait or bait containing imidacloprid.

DISCUSSION

Novel compounds were initially evaluated to find replacements for organophosphates and carbamates. Results of several insecticides warrant future field trials to determine the efficacy of different bait spray formulations for controlling *R. mendax*. Compounds differed in their ability to incapacitate and kill flies. Depending on the insecticide chosen for inclusion in protein baits, the modes of action can be predominantly knockdown (acetamiprid, clothianidin, and imidacloprid) or kill (fipronil and spinosad).

TABLE 3. FLY KNOCKDOWN AND MORTALITY AFTER 4 H EXPOSURE TO BAIT CONTAINING 40 PPM INSECTICIDE.

Treatment	Knockdown (%)	Mortality (%)	
	3 h	24 h	48 h[1]
Clothianidin	11.3 ± 1.3 a	13.8 ± 3.8 a	25.0 ± 6.5
Fipronil	8.8 ± 4.3 a	26.3 ± 9.4 a	37.5 ± 14.4
Imidacloprid	5.0 ± 2.0 a	12.5 ± 4.3 ab	23.8 ± 7.2
Spinosad	3.8 ± 1.3 ab	16.3 ± 2.4 a	35.0 ± 7.4
Control	0.0 ± 0.0 b	3.8 ± 2.4 b	16.3 ± 5.9

Values in the same column having the same letter are not significantly different (Fisher's LSD test, $P = 0.05$).
[1]NS, ANOVA, $P > 0.05$.
$n = 400$ flies.

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TABLE 4. FLY KNOCKDOWN AND MORTALITY AFTER EXPOSURE TO BLUEBERRY LEAVES CONTAINING BAIT DROPLETS WITH 40 PPM INSECTICIDE.

Experiment	Treatment	Knockdown (%)	Mortality (%)	
		1 h	24 h	48 h
Fresh[1]	Acetamiprid	12.0 ± 4.4 a	42.0 ± 6.9 b	68.0 ± 4.4 b
	Clothianidin	24.0 ± 8.3 a	58.0 ± 8.6 b	78.0 ± 4.6 b
	Fipronil	0.0 ± 0.0 b	80.0 ± 4.2 a	96.0 ± 2.6 a
	Imidacloprid	16.0 ± 4.0 a	44.0 ± 4.9 b	68.0 ± 8.5 b
	Spinosad	0.0 ± 0.0 b	80.0 ± 6.6 a	94.0 ± 3.0 a
	Control	0.0 ± 0.0 b	14.0 ± 2.1 c	30.0 ± 9.1 c
1-d-old[1]	Acetamiprid	16.0 ± 4.0 a	30.0 ± 7.4 b	56.0 ± 10.2 b
	Clothianidin	2.0 ± 2.0 bc	28.0 ± 8.0 b	58.0 ± 9.1 b
	Fipronil	0.0 ± 0.0 c	60.0 ± 6.6 a	86.0 ± 5.2 a
	Imidacloprid	6.0 ± 3.0 b	10.0 ± 4.4 b	30.0 ± 7.4 c
	Spinosad	0.0 ± 0.0 c	68.0 ± 6.8 a	88.0 ± 4.4 a
	Control	0.0 ± 0.0 c	16.0 ± 10.2 b	22.0 ± 10.5 c

For each experiment, values in the same column having the same letter are not significantly different (Fisher's LSD test, $P = 0.05$).

[1] $n = 300$ flies.

Bait sprays containing feeding stimulants (e.g., sucrose) have several advantages to conventional sprays. Lower concentrations of insecticide are needed in bait sprays than conventional sprays because mortality is primarily from oral toxicity, which has lower LC50 thresholds than dermal toxicity, and more insecticide is consumed because of the presence of feeding stimulants (e.g., sucrose) (Hu et al. 2000; Reissig 2003; Barry & Polavarapu 2004). Therefore, baits sprays can be applied at a lower rate of active ingredient per hectare than conventional sprays. The attraction and feeding responses of flies to bait sprays have led to evaluations assessing their potential use as border sprays (Prokopy et al. 2003; Prokopy et al. 2004).

Fly survivorship differed based on concentration and type of insecticide used. As expected, higher concentrations of insecticide resulted in higher mortality of flies. At 400 ppm the shortest lag time between feeding and 100% mortality resulted from bait containing fipronil, followed by bait containing spinosad. The pyrethroid deltamethrin did not result in fly knockdown or mortality that was significant enough to warrant further evaluation on *R. mendax*, which was also the finding of Barry et al. (2004) investigating insecticidal coatings for spheres used in attract and kill of *R. mendax*. The neonicotinoids (acetamiprid, clothianidin, and imidacloprid) resulted in intermediate survivorship, performing better than deltamethrin, but not as well as spinosad or fipronil. Our findings are in agreement with Reissig (2003), who found the LC50 (with flies unable to walk considered dead) of imidacloprid and spinosad for *R. pomonella* to be approximately 11 ppm and between 3-10 ppm, respectively.

Many published insecticide assays involve exposing flies to an insecticide treatment for several days in a small container to determine mortality. These conditions are likely to underestimate the concentration of insecticide needed for fly mortality in the field. The importance of such studies is to determine the suitable type and range of activity for insecticides to be further evaluated. In the current study we used three types of assays to evaluate the effects of insecticides: a variable feeding duration (up to 5 min), a fixed short duration (Feeding Assay—10 s), and a fixed long duration (Survivorship Unlimited Access). Each of these assays has limitations, but taken together supports the findings of the other assays.

Sub-lethal effects of insecticides are known to manifest as a reduction in fecundity, measured indirectly from oviposition punctures by *R. pomonella* (Reissig 2003). In most of the assays in the current study, observations for knockdown occurred 1 h after a fly fed, but flies feeding on the neonicotinoids were often in that state much earlier and later, as evidenced by some flies being unable to feed for the duration of the 10-s trial from becoming incapacitated. Liburd et al (2003) found insecticide-fed flies have lower levels of activity compared with a control. In our study flies that were knocked down often died, but some of the flies in this condition appeared no different than control flies after 1-2 d, apparently recovering from exposure to the insecticide. This finding leads us to suggest that there may be an optimal concentration for consumption to achieve the desired mortality.

Measuring fly mortality in the context of field evaluations of insecticides contained in bait

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sprays is one way to determine the effectiveness of knockdown. This would provide a realistic setting in which the effects of natural enemies could be evaluated on flies that are not completely dead, as well as other sub-lethal effects associated with a reduction in oviposition and larval presence. The results of future field trials can determine the effectiveness of bait sprays containing insecticides with different modes of action.

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