

# Comparative Evaluation of Spinosad and Phloxine B as Toxicants in Protein Baits for Suppression of Three Fruit Fly (Diptera: Tephritidae) Species

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**ABSTRACT** Spinosad and phloxine B are two more environmentally friendly alternative toxicants to malathion for use in bait sprays for tephritid fruit fly suppression or eradication programs. Laboratory tests were conducted to assess the relative toxicity of these two toxicants for melon fly, *Bactrocera cucurbitae* Coquillett; oriental fruit fly, *Bactrocera dorsalis* Hendel; and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) females. Field tests also were conducted with all three species to compare these toxicants outdoors under higher light and temperature conditions. In laboratory tests, spinosad was effective at much lower concentrations with LC<sub>50</sub> values at 5 h of 9.16, 9.03, and 4.30 compared with 250.0, 562.1, and 658.9 for phloxine B (27, 62, and 153 times higher) for these three species, respectively. At 16 ppm spinosad, LT<sub>50</sub> values were lower for all three species (significantly lower for *C. capitata* and *B. dorsalis*) than 630 ppm phloxine B LT<sub>50</sub> values. At 6.3 ppm spinosad, the LT<sub>50</sub> value for *C. capitata* (3.94) was still significantly less than the 630 ppm phloxine B LT<sub>50</sub> value (6.33). For all species, the 100 ppm spinosad concentrations gave LT<sub>50</sub> values of <2 h. In comparison among species, *C. capitata* was significantly more sensitive to spinosad than were *B. cucurbitae* or *B. dorsalis*, whereas *B. cucurbitae* was significantly more sensitive to phloxine B than were *C. capitata* or *B. dorsalis*. LC<sub>50</sub> values were reduced for both toxicants in outdoor tests, with greater reductions for phloxine B than for spinosad for *B. dorsalis* and *B. cucurbitae*. Fly behavior, though, is likely to keep flies from being exposed to maximum possible outdoor light intensities. Comparable levels of population suppression for any of the three species tested here will require a much higher concentration of phloxine B than spinosad in the bait.

**KEY WORDS** *Ceratitis capitata*, *Bactrocera dorsalis*, *Bactrocera cucurbitae*, spinosad, phloxine B

TEPHRITID FRUIT FLIES ARE serious pests of fruit crops throughout the world. In Hawaii, four alien tephritid fruit fly species of economic importance have become established: melon fly, *Bactrocera cucurbitae* Coquillett; Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); oriental fruit fly, *Bactrocera dorsalis* Hendel, and *Bactrocera latifrons* Hendel. These four species infest a wide range of fruits and vegetables both in Hawaii and in many other countries (White and Elson-Harris 1992). Introduction of any of these species to agricultural areas where they do not exist may initiate costly eradication programs, such as have been conducted in California and Florida. Suppression programs, in areas where these species are established, and eradication programs in areas of recent invasion, have typically entailed the use of protein bait sprays incorporating malathion. Although these sprays are effective, they are controversial because of concerns for adverse effects on human health and on nontarget

organisms. Two potential more environmentally friendly alternative toxicants for use with protein baits for suppression or eradication programs are spinosad and phloxine B (Peck and McQuate 2000). Spinosad is an insecticidal toxin of two macrocyclic lactones called spinosyns A and D, derived from metabolites of the actinomycete bacterium *Saccharopolyspora spinosa* (Sparks et al. 1998). Phloxine B (2',4',5',7'-tetrabromo-4,5,6,7-tetrachloro-fluorescein, disodium salt) is a photoactive xanthene dye that has insecticidal properties. These two toxicants differ in their mode of action. The spinosyns in spinosad excite neurons in the central nervous system, causing involuntary muscle contractions that lead to paralysis from neuromuscular fatigue (Salgado 1998). Phloxine B, however, transfers absorbed light energy to ground state oxygen, forming singlet oxygen, an effective oxidizing agent, that attacks internal body tissues (Heitz 1997). Prior published results have shown the effectiveness of spinosad for Mediterranean fruit fly (laboratory, Adán et al. 1996, Vargas et al. 2002, Stark et al. 2004; field, Peck and McQuate 2000, Burns et al. 2001, McQuate et al. 2005), for oriental fruit fly (laboratory, Stark et al. 2004), and for melon fly (laboratory, Stark

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et al. 2004; field, Prokopy et al. 2003). Prior published results also have shown the effectiveness of phloxine B for Mediterranean fruit fly (laboratory, Liquido et al. 1995a, Vargas et al. 2002; field, McQuate and Peck 2000, Peck and McQuate 2000, Moreno et al. 2001) and for oriental fruit fly (laboratory, Liquido et al. 1995b, c; field, McQuate et al. 1999).

The effectiveness of suppression with bait sprays is influenced by both attraction and feeding responses of the flies. Such responses can be affected by both the type of protein in the bait and the toxicant used (Liquido et al. 1995a, Vargas et al. 2002). It has been found that neither spinosad nor phloxine B cause repellency of the bait to protein-starved Mediterranean fruit flies, whereas a bait incorporating malathion was found to be repellent (Vargas et al. 2002). Similar tests have not yet been reported for melon fly or oriental fruit fly.

There has, however, been no publications that present comparative laboratory toxicological bioassays of both phloxine B and spinosad among different tephritid fruit fly species. Here, we present results of laboratory bioassays with Mediterranean fruit fly, melon fly, and oriental fruit fly (we were not able to include *Bactrocera latifrons* in these tests) that compare the effectiveness of phloxine B and spinosad as toxicants in protein bait solutions. Because the effectiveness of photoactive dyes increases with increased light intensity (Fondren and Heitz 1978; Clement et al. 1980; G.T.M., unpublished data) and is affected by light source (Fondren and Heitz 1979), we also present results of open-field bioassays conducted for all three fruit fly species under mid-day ambient conditions to compare the effectiveness of phloxine B and spinosad in higher illumination sunlit conditions.

### Materials and Methods

**Flies.** Mediterranean fruit flies, oriental fruit flies, and melon flies were obtained as pupae from laboratory colonies at the U.S. Pacific Basin Agricultural Research Center in Honolulu, HI. These colonies had been maintained for >30, 150, and 300 generations, respectively. Fruit flies used in our tests were kept in an insectary at 24–27°C, 65–70% RH, and a photoperiod of 12:12 (L:D) h. Adults were fed water and a diet consisting of three parts sucrose, one part protein yeast hydrolysate (Enzymatic, U.S. Biochemical Corporation, Cleveland, OH), and 0.5 part torula yeast (Lake States Division, Rhineland Paper Co., Rhineland, WI) from the time of emergence from puparia until noon the day before the experiment, at which time sets of 20 females were placed in separate feeding chambers (2-liter transparent plastic cups; Sweetheart Cup Co., Chicago, IL), each covered with a single layer of organza secured by a rubber band, and held with water only until the start of the experiment. Flies were 3–5 d old at the start of the experiments.

**Chemicals.** An aqueous suspension of spinosad [NAF-315, lot #MB1116OP21, 22.8% (wt:wt) active ingredient] was obtained from Dow Agrosciences (Indianapolis, IN). Phloxine B (94% purity) was obtained from Hilton Davis Co. (Newark, NJ). These toxicants

were mixed in a protein bait solution similar to that developed by Moreno et al. (2001) for field use with tephritid fruit flies: 70% (vol:vol) Mazoferm E802 (Corn Products, Argo, IL), 20% (vol:vol) invertose (LSI, Emeryville, CA), 2.0% (vol:vol) polyethylene glycol 200 (ICN Biomedicals Inc., Aurora, OH), 1.0% (vol:vol) Tween 60 (ICN Biomedicals Inc.), 1.0% (wt:vol) soybean oil (Aldrich, Milwaukee, WI), and 0.6% (wt:vol) ammonium acetate (Sigma, St. Louis, MO). The remaining 6.0% (vol:vol) was composed of water and the toxicant, with water concentration decreased to counter increased toxicant concentrations.

**Preparation of Bait Solutions.** *Spinosad.* Bait solutions with spinosad (AI) concentrations of 0.0, 1.0, 2.5, 6.3, 16.0, 40.0, and 100.0 ppm (wt:wt) were prepared by first making an aqueous dilution containing 0.005 g of spinosad (AI) per 1.0 g of solution (King and Hennessey 1996). Then, appropriate quantities of this dilution and water were added to establish the spinosad concentrations indicated above. The spinosad concentrations used encompass that used in a fruit fly bait recently marketed by Dow Agrosciences (GF-120 Fruit Fly Bait, 80 ppm when the bait is diluted according to label directions).

*Phloxine B.* Bait solutions with phloxine B (AI) concentrations of 0.0, 100.0, 160.0, 250.0, 400.0, 630.0, and 1000.0 ppm (wt:wt) were prepared.

Toxicant concentration ranges for both toxicants used were selected based on preliminary research, with specific concentrations selected so as to provide uniform intervals based on a log<sub>10</sub> transformation of the concentrations.

**Laboratory Toxicity Bioassays.** Previous research trials had compared the effectiveness of both phloxine B and spinosad between males and females and found similar mortality response (G.T.M., unpublished data). Stark et al. (2004) found males and females to be equally susceptible to spinosad for both oriental fruit fly and melon fly, although they found male Mediterranean fruit fly slightly more susceptible to spinosad than females. Females were selected for testing in the bioassays reported here, because fruit/vegetable damage by adults is caused directly only by females.

On the morning of the test, 80  $\mu$ l (eight 10- $\mu$ l drops) of each of the bait solutions (six with different concentrations of spinosad, six with different concentrations of phloxine B, and one toxicant-free [bait only] control) was added to 1.0 by 3.0-cm sections of Kimwipes EX-L (Kimberly-Clark, Roswell, GA) on 22 by 38-mm plastic feeding boards. The Kimwipes section kept the bait solution droplets from dripping off the feeding board with minimal absorption by the tissue. All feeding boards for the experiment were treated before insertion of any boards into the feeding chambers (2-liter transparent plastic cups; Sweetheart Cup Co.) to minimize the time difference between first and last insertions. Each feeding chamber held 20 female flies. Feeding boards were all inserted by 8:00 a.m. through a hole in the side of the experimental chambers. After 4 h, feeding boards were removed and normal adult maintenance food (as described under the section on "Flies" above) was added to each of the

feeding chambers. Tests were run in separate weeks for Mediterranean fruit fly, oriental fruit fly, and melon fly. Four cool white fluorescent lights (Slimline, General Electric, Cleveland, OH), situated immediately above the chambers, were turned on immediately before feeding boards were inserted in the experimental chambers. Light intensity was measured in lux by using an Extech Foot Candle/Lux Meter (Extech Instruments Corporation, Waltham, MA). The peak sensitivity of this light meter encompasses the wavelength of peak absorption by phloxine B (548 nm; Green 1990). The fluorescent lights provided an average ( $\pm$ SEM) light intensity of  $19,492 \pm 179$  (range 15,300–22,400),  $14,727 \pm 96$  (range 12,980–16,500), and  $12,599 \pm 95$  (range 11,000–14,280) lux measured just below the organza, at the middle of the chamber, and at the bottom of the chamber, respectively, throughout the light phase of the bioassay. The lights were turned off at 6:00 p.m. Thereafter, a photoperiod of 12:12 (L:D) h was maintained, with lights turned on at 6:00 a.m. Throughout the trials, average temperature ( $\pm$ SEM) was  $25.3 \pm 0.01^\circ\text{C}$  (range 23.4–28.7) and average relative humidity ( $\pm$ SEM) was  $37.2 \pm 0.47\%$  (range 29.0–51.2). A continual supply of water was maintained throughout the test through wicks saturated with tap water inserted through the bottom of each of the chambers. Mortality of flies was recorded at the beginning of the feeding period, every hour until 4:00 p.m. of day 1, every 2 h from 8:00 a.m. to 4:00 p.m. on day 2, and at 8:00 a.m. on day 3, at which point the test was terminated, 48 h after the introduction of the feeding boards. During each test, there were four chambers of each treatment, organized in a randomized block design. The test was conducted three times for each species.

**Outdoor Toxicity Bioassay.** The outdoor bioassay used the same chambers, same feeding boards, and same number of female flies per chamber as described above for the laboratory tests. The chambers were set outside in an open grassy field at the USDA-ARS-PBARC laboratory in Hilo, HI, on four 3.5 by 0.24-m boards organized in a square design, held 42 cm above the ground on concrete blocks. The range of toxicant concentrations tested was changed to accommodate higher mortality rates, and mortality counts were maintained for only 5 h with the trial starting at 9:45 a.m. rather than at 8:00 a.m. Concentrations used were 0, 1, 2, 4, 8, and 16 ppm for spinosad and 0, 10, 20, 40, 80, and 160 ppm for phloxine B. A run time of 5 h was selected because the 5-h laboratory data provided the best data for the calculation of  $LC_{50}$  values through probit analysis, and this test was intended to provide a comparison between  $LC_{50}$  values resulting under the two different light conditions. The experiment's later start time was chosen so that the 5-h test would occur over the times of highest outdoor light intensity. The outdoor trial was run on separate days for the three fruit fly species. Over the 5-h trial, the average temperature, relative humidity, and light intensity directly under the organza was  $30.0 \pm 0.65^\circ\text{C}$  (range 25.2–42.0),  $53.5 \pm 1.11\%$  RH (range 39.6–65.3), and  $82,042 \pm 6290$  lux (range 38,500–147,700) for oriental

fruit fly;  $28.1 \pm 0.23^\circ\text{C}$  (range 25.8–29.3),  $58.6 \pm 0.58\%$  RH (range 53.8–65.1), and  $68,258 \pm 5820$  lux (range 21,100–115,500) for melon fly; and  $27.7 \pm 0.36^\circ\text{C}$  (range 24.6–29.5),  $47.8 \pm 0.71\%$  RH (range 43.6–55.0), and  $103,621 \pm 4732$  lux (range 38,500–134,200) for Mediterranean fruit fly. The days of the outdoor tests were mostly sunny with passing clouds. These tests were intended to provide a suggestion of higher mortalities that might be found under conditions of the higher illumination possible under outdoor ambient conditions. Analysis of the effect of a range of levels of sustained outdoor light intensities is beyond the scope of this study.

**Statistical Analysis.** Toxicity of spinosad and phloxine B to adult tephritid fruit flies 5 h after initial exposure to baits with and without toxicant was analyzed, for both indoor and outdoor tests, by using probit analyses in POLO-PC (LeOra Software 1987). POLO-PC uses Abbott's formula (Abbott 1925) to correct for control mortality.  $LC_{50}$  and  $LC_{90}$  values were calculated based on 5-h mortality data because that data gave good probit regressions for both toxicants for all species, whereas regressions were poor at longer time periods because of 100% mortalities at higher toxicant concentrations.  $LT_{50}$  and  $LT_{90}$  values were calculated based on mortalities from 0 to 48 h.

Differences among fly species were considered significant when there was no overlap of their respective 95% fiducial limits (FL) of the  $LC_{50}$ ,  $LC_{90}$ ,  $LT_{50}$ , or  $LT_{90}$  values. Tests of parallelism and equality of probit regression lines for all species pairs also were made with POLO-PC by using  $\chi^2$  goodness-of-fit tests.

## Results

**Laboratory Tests.** Control mortalities for the probit analyses were 0.0% for melon flies and oriental fruit flies and 2.1% for Mediterranean fruit flies. Average percentage of mortalities over the range of tested concentrations of spinosad and phloxine B are presented in Fig. 1 for melon fly, Fig. 2 for oriental fruit fly, and Fig. 3 for Mediterranean fruit fly. These charts give a good visual image of the quicker kill rates with spinosad for all three species, even with significantly lower concentrations than the phloxine B treatments.  $LC_{50}$  and  $LC_{90}$  values for each toxicant and for each species are presented in Table 1.  $LT_{50}$  and  $LT_{90}$  values are presented in Table 2 for each species for spinosad concentrations of 6.3, 16, and 100 ppm and phloxine B concentrations of 250 and 630 ppm. These concentrations were chosen because they provide a good comparison of the relation of concentration of the two toxicants to toxicity and because the 100 ppm spinosad concentration is close to the 80 ppm spinosad concentration in a commercially available fruit fly bait (GF-120 Fruit Fly Bait, Dow Agrosciences LLC), when diluted according to label directions.

For melon fly, oriental fruit fly, and Mediterranean fruit fly, the  $LC_{50}$  for phloxine B was 27, 62, and 153 times that of the  $LC_{50}$  for spinosad, respectively. The  $LC_{90}$  for phloxine B was 30, 51, and 91 times that of the  $LC_{90}$  for spinosad, respectively.

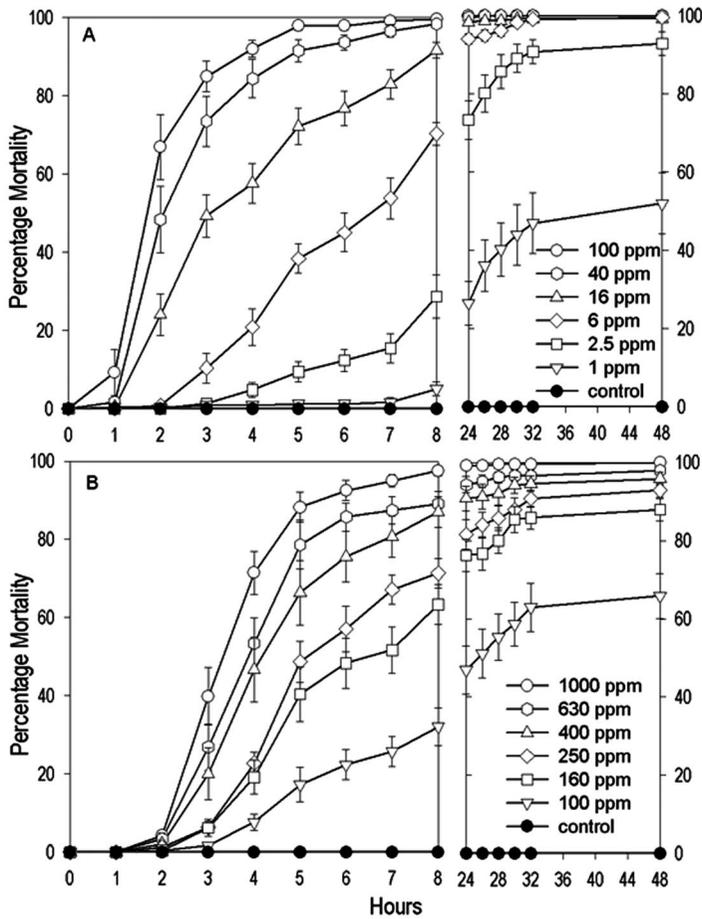


Fig. 1. Average percentage of mortality ( $\pm$ SEM) of adult melon flies after exposure to bait solutions with different concentrations of (A) spinosad and (B) phloxine B.

Equality of slopes of probit regressions was rejected for all species pairs with phloxine B and with all pairs except oriental fruit fly and melon fly for spinosad. Parallelism of slopes of probit regressions was rejected for all species pairs with phloxine B but could not be rejected for any species pairs with spinosad (Table 3; Fig. 4).

**Outdoor Test.**  $LC_{50}$  and  $LC_{90}$  values for each toxicant and each species in the outdoor tests are presented in Table 1. With the increased light and temperature outdoors, the  $LC_{50}$  values for spinosad for all species dropped to <50% of the indoor  $LC_{50}$ . The percentage decrease was similar for all three species, with the outdoor  $LC_{50}$  35, 35, and 44% of the indoor  $LC_{50}$  for melon fly, oriental fruit fly, and Mediterranean fruit fly, respectively. The change, though, was more variable for phloxine B, with the outdoor  $LC_{50}$  36, 6, and 10% of the laboratory  $LC_{50}$  for melon fly, oriental fruit fly, and Mediterranean fruit fly, respectively. The  $LC_{50}$  for phloxine B in the outdoor test was 28, 9.7, and 36 times that of the  $LC_{50}$  for spinosad, compared with 27, 62, and 153 times in the laboratory test.

### Discussion

For spinosad, the  $LC_{50}$  for Mediterranean fruit fly was significantly lower than that for melon fly or for oriental fruit fly. There was, however, no significant difference in  $LC_{50}$  between melon fly and oriental fruit fly (Table 1). By 24 h, there was 100% mortality at the highest concentration of spinosad (100 ppm) for all three fruit fly species, which was not the case for phloxine B (see below). Our results differ somewhat from those of Stark et al. (2004). Our reported  $LC_{50}$  values are significantly higher for oriental fruit fly and melon fly, as would be expected because our numbers are based on 5 h, whereas the values presented in Stark et al. (2004) are based on 24 h. Our reported  $LC_{50}$  value for Mediterranean fruit fly (4.30), however, was very similar to that reported by Stark et al. (2004) (4.2). The 24 h  $LC_{50}$  for spinosad for oriental fruit fly was significantly lower than for melon fly or for Mediterranean fruit fly in Stark et al. (2004), but, in our study, the trend in mortality between 5 and 24 h increased more for melon fly than for oriental fruit fly (Figs. 1 and 2), which would lead to melon fly having

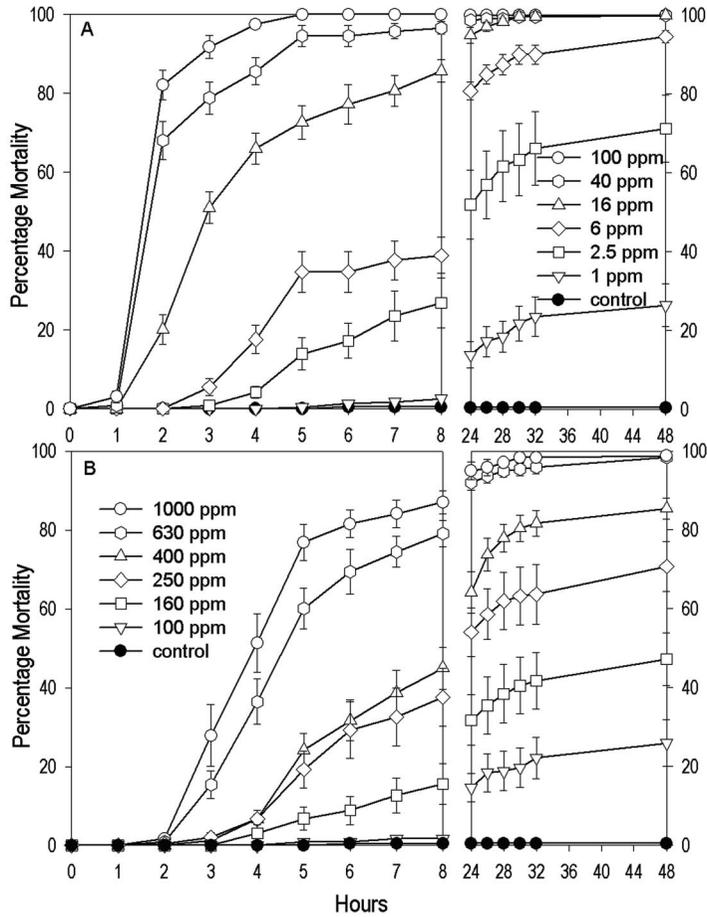


Fig. 2. Average percentage of mortality ( $\pm$ SEM) of adult oriental fruit flies after exposure to bait solutions with different concentrations of (A) spinosad and (B) phloxine B.

a numerically lower  $LC_{50}$  at 24 h. Overall, though, our results for female response to spinosad are generally comparable with those of Stark et al. (2004), and differences may be attributable to differences in temperature and relative humidity and the use of different protein baits for the toxicity tests. The tests by Stark et al. (2004) were based on Provesta 621 (Integrated Ingredients, Bartlesville, OK), whereas our tests were based on Mazoferm E802, with a number of additional ingredients.

For phloxine B, the  $LC_{50}$  for melon fly was significantly lower than that for Mediterranean fruit fly or for oriental fruit fly. There was, however, no difference in  $LC_{50}$  between Mediterranean fruit fly and oriental fruit fly (Table 1). For phloxine B, mortality at the highest concentration (1000 ppm) failed to reach 100% at 24 h or even 48 h for Mediterranean fruit fly and oriental fruit fly, whereas mortality reached 100% at 48 h, but not 24 h, for melon fly. These results, together with the outdoor  $LC_{50}$  results, indicate that phloxine B concentration needs to be at least 10-fold greater than spinosad concentration for comparable mortality rates.

It is interesting to note that the order of effectiveness among the three species of fruit fly for phloxine B was reversed from that for spinosad. For example, melon flies are more susceptible to phloxine B than are the other species, but they are the least susceptible to spinosad among the three species (although not significantly different from oriental fruit fly), based on the bait system used in this study. The different order of effectiveness may be reflective of the different modes of action of the toxicants. For phloxine B, mortality requires exposure to light. Mortality among the three species to the phloxine B-containing bait increased as the size of the fly increased. Using flies from the same source as used in our toxicity tests, we weighed 50 flies of each fly species. The average weights were 0.0054, 0.0091, and 0.011 g for Mediterranean fruit fly, oriental fruit fly, and melon fly, respectively, with the square-root transformed weights significantly different for each species ( $F = 342.7$ ;  $df = 2, 147$ ;  $P < 0.0001$ , with a Tukey-Kramer test for mean separation). Melon fly is significantly larger than the other two species, and its phloxine B  $LC_{50}$  was significantly less. Perhaps the greater surface area of the larger flies provided greater opportunity for light penetration,

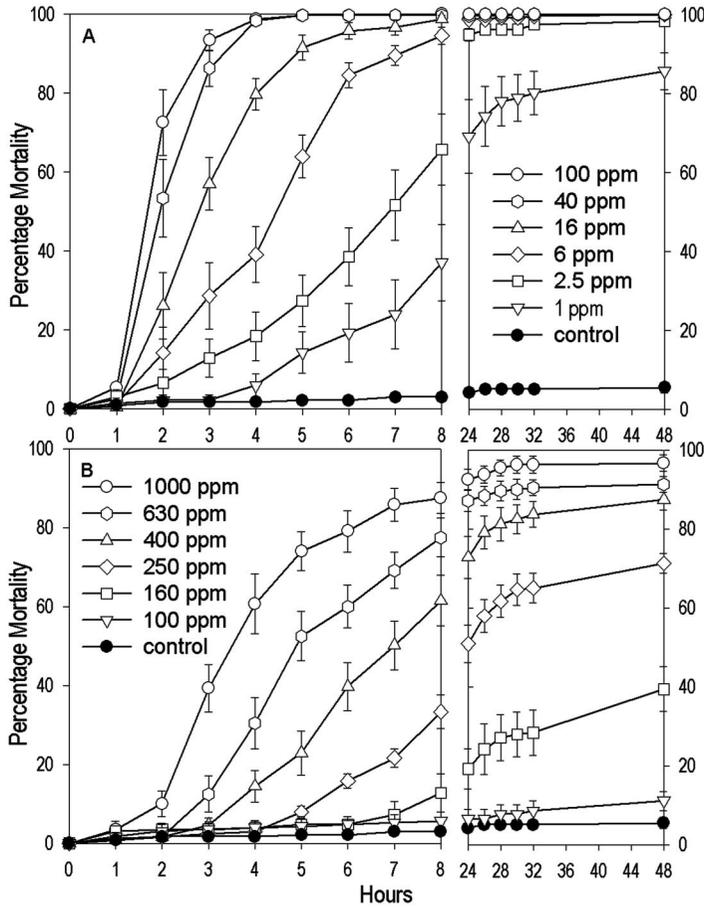


Fig. 3. Average percentage of mortality ( $\pm$ SEM) of adult Mediterranean fruit flies after exposure to bait solutions with different concentrations of (A) spinosad and (B) phloxine B.

leading to greater activation of the dye. However, for spinosad, mortality is based strictly on consumption. If it would take the larger flies longer to consume comparable quantities, mortality rates could be reduced and that was the order of mortality observed. However, although these explanations fit the trends, it should be pointed out

that not all differences in  $LC_{50}$  were significant, and we have no data to document the actual quantities and/or rates of bait (or toxicant) consumed.

Change in activity of phloxine B per unit change in dose was not the same for any of the species pairs for phloxine B. Change in activity of spinosad per unit

Table 1. Calculated 5-h  $LC_{50}$  and  $LC_{90}$  values and slopes based on probit analyses. Values in the  $LC_{50}$  and  $LC_{90}$  columns followed by the same letter are not significantly different at the  $\alpha = 0.05$  level, with statistical comparisons made separately for each toxicant

Toxicant	Site	Fly species	n	Slope $\pm$ SE	$LC_{50}$ ppm (95% FL)	$LC_{90}$ ppm (95% FL)
Spinosad	Indoor	<i>B. cucurbitae</i>	1,432	2.19 $\pm$ 0.094	9.16 (8.34–10.06)c	35.27 (30.77–41.17)c
	Indoor	<i>B. dorsalis</i>	1,432	2.45 $\pm$ 0.11	9.03 (7.07–11.56)c	30.49 (22.14–48.38)bc
	Indoor	<i>C. capitata</i>	1,419	2.24 $\pm$ 0.12	4.30 (3.10–5.61)b	16.08 (11.76–25.33)ab
	Outdoor	<i>B. cucurbitae</i>	320	2.29 $\pm$ 0.29	3.23 (1.72–4.63)*ab	11.71 (7.74–29.81)*a
	Outdoor	<i>B. dorsalis</i>	301	3.15 $\pm$ 0.38	3.20 (1.81–4.48)*ab	8.15 (5.70–17.29)*a
	Outdoor	<i>C. capitata</i>	315	1.90 $\pm$ 0.36	1.88 (0.96–2.70)a	8.86 (6.47–14.87)a
Phloxine B	Indoor	<i>B. cucurbitae</i>	1,426	2.03 $\pm$ 0.11	250.00 (215.47–286.63)d	1069.67 (841.95–1492.43)b
	Indoor	<i>B. dorsalis</i>	1,428	2.91 $\pm$ 0.15	562.07 (471.70–698.11)e	1549.35 (1132.55–2594.88)b
	Indoor	<i>C. capitata</i>	1,425	3.15 $\pm$ 0.20	658.88 (617.94–704.16)e	1469.84 (1297.09–1729.74)b
	Outdoor	<i>B. cucurbitae</i>	320	2.40 $\pm$ 0.42	90.57 (71.28–111.96)c	310.09 (111.96–219.87)a
	Outdoor	<i>B. dorsalis</i>	284	2.54 $\pm$ 0.34	30.97 (14.83–45.89)*ab	99.16 (64.63–278.47)*a
	Outdoor	<i>C. capitata</i>	318	3.68 $\pm$ 0.52	68.43 (40.43–94.86)*b	152.42 (108.17–336.28)*a

\* 90% FL values are listed for some of the outdoor tests because index of significance for potency estimates (g) exceeded 0.5 for 95% level of significance).

**Table 2. Calculated LT<sub>50</sub> and LT<sub>90</sub> values (hours) and slopes based on probit analyses**

Toxicant	Fly species	Concn (ppm)	n	Slope ± SE	LT <sub>50</sub> ppm (95% FL)	LT <sub>90</sub> ppm (95% FL)
Spinosad	<i>B. cucurbitae</i>	6.3	3346	3.25 ± 0.098	6.72 (6.19–7.30)f	16.66 (14.79–19.21)fg
		16	3374	2.88 ± 0.10	3.39 (2.90–3.89)c	9.47 (7.95–11.93)d
		100	3346	4.36 ± 0.19	1.78 (1.65–1.90)a	3.50 (3.27–3.78)b
	<i>B. dorsalis</i>	6.3	3346	2.56 ± 0.070	9.94 (9.13–10.81)g	31.54 (27.84–36.45)i
		16	3346	2.69 ± 0.095	3.42 (2.83–4.01)bc	10.22 (8.37–13.41)de
		100	3346	6.51 ± 0.33	1.67 (1.51–1.83)a	2.63 (2.39–2.98)a
	<i>C. capitata</i>	6.3	3318	3.67 ± 0.14	3.94 (3.31–4.59)cd	8.81 (7.21–10.02)d
		16	3276	5.04 ± 0.21	2.77 (2.66–2.89)b	4.98 (4.76–5.23)c
		100	3360	6.04 ± 0.29	1.69 (1.50–1.88)a	2.76 (2.47–3.19)a
Phloxine B	<i>B. cucurbitae</i>	250	3318	2.15 ± 0.063	7.30 (5.58–9.30)fg	28.79 (20.79–46.83)hi
		630	3290	2.64 ± 0.086	3.96 (2.73–5.28)bcde	12.09 (8.67–21.26)defgh
		250	3346	1.73 ± 0.060	18.67 (15.39–23.28)h	103.18 (70.42–179.04)j
	<i>B. dorsalis</i>	630	3290	2.64 ± 0.080	5.34 (4.30–6.50)def	16.38 (12.75–23.37)defgh
		250	3360	1.95 ± 0.066	20.77 (18.04–24.10)h	94.01 (71.92–133.29)j
		630	3360	2.13 ± 0.063	6.33 (4.84–8.04)ef	25.29 (18.47–40.14)ghi

Values in the LT<sub>50</sub> and LT<sub>90</sub> columns followed by the same letter are not significantly different at the α = 0.05 level, with statistical comparisons made across toxicants, species, and concentrations.

change in dose was comparable between all different species pairs, with the test for equality of probit regression lines not able to be rejected for the oriental fruit fly–melon fly pairing (Table 3), suggesting that these two species are the most similar in regard to their response to spinosad.

High mortality rates were found with much lower concentrations of spinosad than phloxine B for the tests conducted under fluorescent lights. LC<sub>50</sub> values for phloxine B ranged from 27 times (melon fly) to 153 times (Mediterranean fruit fly) greater than those for spinosad (Table 1). These ratios, however, would be expected to decrease when the light intensity is increased, which was seen in the outdoor test with oriental fruit fly and Mediterranean fruit fly where LC<sub>50</sub> values for both phloxine B and spinosad decreased, but the decrease for phloxine B was much greater than for spinosad. It was surprising that the outside decrease in LC<sub>50</sub> was about the same for phloxine B and spinosad for melon fly, which may relate to some behavioral difference in feeding response under open air conditions.

LT<sub>50</sub> values help to further compare the effectiveness of spinosad and phloxine B. At 16 ppm spinosad, LT<sub>50</sub> values were lower for all three species (significantly lower for Mediterranean fruit fly and oriental fruit fly) than 630 ppm phloxine B LT<sub>50</sub> values. At 6.3 ppm spinosad, the LT<sub>50</sub> value for Mediterranean fruit fly was still significantly less than the 630 ppm (hundred-fold higher concentration) phloxine B LT<sub>50</sub> value. However, the 6.3 ppm spinosad LT<sub>50</sub> values for

the other two species were both significantly higher than their respective 630 ppm phloxine B LT<sub>50</sub> values. For all species, the 100 ppm spinosad concentrations gave LT<sub>50</sub> values <2 h.

The observed decrease in LC<sub>50</sub> values for spinosad for all three species in the outside tests was not expected because light intensity is not known to effect the efficacy of spinosad. However, in all outdoor tests, average temperature was greater than in the laboratory tests and increase in efficacy of spinosad has been reported as temperature increases (Amarasekare and Edelson 2004). Whether it was strictly a temperature effect or was based on some other physiological response to the altered environmental conditions, it is interesting to note that the percentage change between inside and outside spinosad LC<sub>50</sub> values was very similar for all three species.

Overall, comparison of the effectiveness of spinosad and phloxine B is complicated by uncertainty as to the light intensity to which tephritid fruit flies would be exposed in the field after consumption of a protein bait. Light intensity varies with time of year and time of day as well as location within the canopy. Hendrichs and Hendrichs (1990), in a semi-isolated mixed orchard in southern Egypt in early autumn, observed that Mediterranean fruit flies began the day in upper tree canopies that are exposed to the rising sun. Flies then moved progressively down in the canopy to more shaded locations as light intensity and temperature increased. By midday, flies had moved to the interior portions of the lower part of the canopy and tended to

**Table 3. Results of χ<sup>2</sup> tests for equality and parallelism of lines of probit regressions for *B. cucurbitae*, *B. dorsalis*, and *C. capitata* for the toxicants spinosad and phloxine B**

Toxicant	Fruit fly species comparison	Test for line equality				Test for parallelism of lines			
		χ <sup>2</sup>	df	P	Equal?	χ <sup>2</sup>	df	P	Parallel?
Spinosad	<i>B. dorsalis</i> – <i>B. cucurbitae</i>	2.8	2	0.243	Yes	2.77	1	0.096	Yes
	<i>C. capitata</i> – <i>B. cucurbitae</i>	115.0	2	0.000	No	0.10	1	0.752	Yes
	<i>C. capitata</i> – <i>B. dorsalis</i>	116.2	2	0.000	No	1.48	1	0.224	Yes
Phloxine B	<i>B. dorsalis</i> – <i>B. cucurbitae</i>	287.1	2	0.000	No	22.99	1	0.000	No
	<i>C. capitata</i> – <i>B. cucurbitae</i>	423.4	2	0.000	No	45.10	1	0.000	No
	<i>C. capitata</i> – <i>B. dorsalis</i>	27.2	2	0.000	No	7.60	1	0.006	No

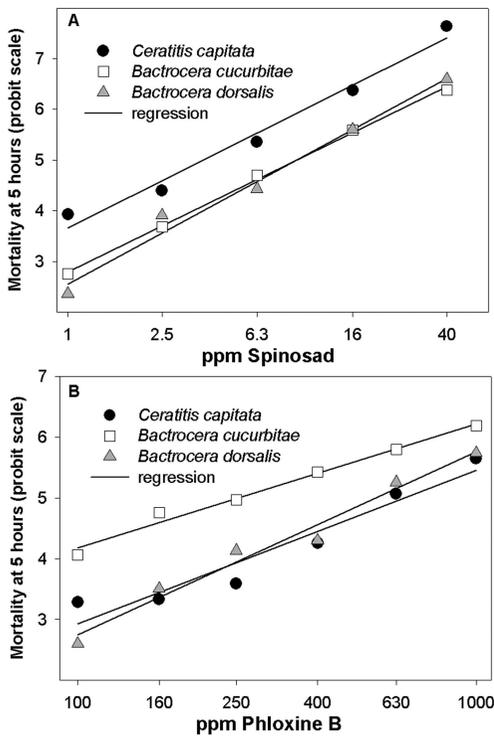


Fig. 4. Five-hour probit regressions for *B. cucurbitae*, *B. dorsalis*, and *C. capitata* for (A) spinosad and (B) phloxine B. See Table 1 for slopes of lines and Table 3 for tests for equality of lines and tests for parallelism of lines.

move to areas of denser canopy. Toward the evening, this trend reversed, with flies progressively moving to the upper part of the canopy (Hendrichs and Hendrichs 1990). If flies favor shaded locations after consumption of baits, the toxic effects of phloxine B would be lessened to a greater extent than with spinosad. However, even very low light levels in the canopy on cloudy days seem to be adequate to maintain some level of photodynamic action with phloxine B, at least during midday hours.

Although effectiveness of phloxine B will depend on the level of light exposure, phloxine B has been shown to be an effective toxicant in protein bait sprays under field conditions. In a field trial comparing the effectiveness of spinosad (100 ppm) and phloxine B (5000 ppm) in a bait spray against Mediterranean fruit flies, there was little difference in effectiveness between these two toxicants, both providing good levels of population suppression (Peck and McQuate 2000). Comparable levels of population suppression, though, for any of the three species tested here, will require a much higher concentration of phloxine B in a bait than the concentration of spinosad, to achieve a comparable mortality rate. The  $LC_{50}$  for phloxine B in the outside test with oriental fruit fly was almost 10 times higher than the  $LC_{50}$  for spinosad, and this concentration difference increased to 28 and 36 times higher for melon fly and Mediterranean fruit fly, respectively. The effectiveness of spinosad at very low concentra-

tions, its apparent low impact on tephritid fruit fly parasitoids (Stark et al. 2004, McQuate et al. 2005), and its availability in a commercial bait formulation (no phloxine B-based products are currently registered for use in the United States) all make spinosad-based bait sprays attractive for suppression of tephritid fruit flies compared with other currently available bait spray products.

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