Oral and Topical Toxicity of Fipronil to Melon Fly and Oriental Fruit Fly (Diptera: Tephritidae)[1]

John D. Stark,[2] Roger Vargas,[3] Neil Miller,[3] and Nancy Chaney[3]

Washington State University, Puyallup Research and Extension Center, Purallup, Washington 98371 USA

J. Entomol. Sci. 44(4): 308-313 (October 2009)

Abstract The objective of this study was to develop oral and topical toxicity data for fipronil in Solulys protein bait to wild melon fly, *Bactrocera cucurbitae* (Coquillett), and the oriental fruit fly, *Bactrocera dorsalis* (Hendel). For the oral study, both females and males were evaluated, whereas in the contact study only females were evaluated. The 24 h oral LC50 estimates for female *B. cucurbitae* and *B. dorsalis* were 113 and 108 mg ai/l, respectively. Female *B. cucurbitae* were more susceptible than males, but female and male *B. dorsalis* were equally susceptible to fipronil after the oral route of exposure. Female *B. cucurbitae* were significantly less susceptible to the fipronil-bait mixture after topical exposure compared with feeding exposure. However, female *B. dorsalis* were equally susceptible to either route of exposure. At the LC50, *B. dorsalis* was significantly more susceptible than *B. cucurbitae* by the topical route of exposure. At the LC90, *B. dorsalis* was significantly more susceptible than *B. cucurbitae* by both oral and topical routes of exposure. Results of this study indicate that there are differences in susceptibility between *B. cucurbitae* and *B. dorsalis* to fipronil, especially at the LC90. *Bactrocera dorsalis* was more susceptible to fipronil than *B. cucurbitae* by oral and topical routes of exposure. LC90 estimates were significantly lower than the 5,333 mg ai/l applied to Amulet Attract and Kill Stations for control of *B. cucurbitae* and *B. dorsalis*.

Key Words melon fly, oriental fruit fly, fipronil toxicity

Tephritid fruit flies (Diptera: Tephritidae) are important pests that cause economic damage to many fruits and vegetables throughout much of the subtropical and tropical areas of the world (Robinson and Hooper 1989). As such, elaborate measures are taken to control or eradicate these species (Peck and McQuate 2000, Chandler 2004). This is particularly true in California which is vulnerable to the establishment of tephritid fruit flies because of the diversity and value of its agricultural products.

A large-scale monitoring program is continually conducted in California to detect introductions of various tephritids including the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *Bactrocera cucurbitae* (Coquillett) (Stark et al. 2004). Detection of tephritids results in the implementation of eradication programs which usually consist of a pesticide spray program (bait-spray) and the release of sterilized male flies (Jackson and Lee 1985). Malathion in protein bait spray has been the most commonly-used insecticide in eradication programs in California (Steiner 1961, Roessler 1989). However, there are

[1]Received 20 August 2008; accepted for publication 28 December 2008.
[2]Address inquiries (email: starkj@wsu.edu).
[3]USDA-ARS, Hilo, HI 96720.

negative consequences associated with the wide-spread application of malathion bait sprays (Ehler and Endicott 1984, Daane et al. 1990, Hoelmer and Dahlsten 1993). Therefore, more environmentally acceptable pesticides are needed for control and eradication of tephritids (Stark et al. 1990, Purcell et al. 1994, Stark et al. 2004).

Two new insecticides that have shown promise for control of tephritid fruit flies are spinosad and fipronil. Spinosad bait spray (GF-120- Dow AgroSciences, Indianapolis, IN) is an effective control of tephritids (Burns et al. 2001, Barry et al. 2003) with extremely high toxicity and low environmental impact (Stark and Vargas 2003). Fipronil is a relatively new insecticide that is being used to control tephritid fruit flies, particularly in the Pacific Island nations (Vargas et al. 2005). Fipronil also has been shown to be efficacious as a control of the blueberry maggot, *Rhagoletis mendax* Curran (Barry et al. 2004). Fipronil has been incorporated into cue-lure (Amulet C-L) and methyl eugenol (Amulet ME) bait stations for control of tephritids (Vargas et al. 2005). However, basic toxicity data for fipronil and tephritid fruit flies have not been published. The objective of this study was to develop oral and topical toxicity data for fipronil in solulys bait spray for 2 *Bactrocera* fruit flies, the melon fly, *B. cucurbitae*, and the oriental fruit fly, *B. dorsalis*.

Materials and Methods

Flies. All flies used in these studies were F1 progeny of wild *B. cucurbitae* and *B. dorsalis* collected from papaya obtained from the Kapoho area of the Island of Hawaii. Parental flies were allowed to oviposit onto papaya which were then placed in plastic containers (8.5 × 18 × 28 cm) containing moist artificial diet (Vargas et al. 1983). The plastic containers were placed in fiberglass tubs (16 × 32 × 49 cm) containing 3 mm of dry sand. Larvae pupated in the sand contained in the fiberglass tubs and were removed from the sand by sieving. Pupae were held in cups containing moist sand inside of screened wooden cages (25 × 25 × 25 cm) until eclosion. The flies were provided with water ad libitum from plastic cups (4 cm deep × 9 cm diam) with a cotton dental wick inserted through a lid, and fed a diet of sugar only. Flies were held in a room maintained at 22 ± 3°C (ambient temperature) and 40 - 90% RH under a L12:D12 h photoperiod.

Oral toxicity study. Batches of protein-starved female and male *B. cucurbitae* and *B. dorsalis* flies, 25 per sex, were placed in wooden cages (25 × 25 × 25 cm) 5 - 6 d after eclosion. Flies were provided with water and sugar throughout the test and held at 21 - 23°C and 75 - 85% RH. Before each replicate test a protein bait solution was prepared by diluting a stock solution of Solulys AST bait (Roquette America INC., Keokuk, IA). Bait solution was stirred by hand for approx. 5 min. prior to testing. Fipronil (Regent 200 SC, 200 g ai/L, BASF Agricultural Products, Research Triangle Park, NC) was diluted with 10% Solulys AST protein bait in water (1 part solulys:9 parts water) to create a range of concentrations for the oral and topical toxicity tests. A stock solution of fipronil was diluted to an initial concentration of 1000 mg ai/l in distilled water. This solution was then stirred for 30 min using a stir rod and a magnetic stirring table. The protein bait solution was then used to make further dilutions of the fipronil-Solulys solution. Bait/toxicant solutions were applied to coffee leaves placed in Petri plates (1.5 cm tall × 9 cm diam). Ten, 10-µL drops were placed on each of 2 leaf strips (2 × 5 cm) with a repeating micropipette. The Petri plates were placed in the cages with the flies for the length of the assay (72 h). After 72 h, the numbers of dead and living flies were counted in each cage. This study was replicated a minimum of 3 times.

Initial tests for all species were conducted with brackets of the following concentrations: 0, 1, 10, 100 and 1000 mg active ingredient (ai)/l. After the initial range finding test, a series of 4 - 6 concentrations was evaluated for each species and sex.

Topical toxicity study. Contact toxicity studies were conducted with fipronil and female *B. cucurbitae* and *B. dorsalis*. Treatments were applied to individual females using a microapplicator (Hamilton, Reno, NV) with a 0.5-ml glass syringe with a 22-gauge needle. For each replicate, batches of 10 female flies were treated with a 1-µL drop of each treatment applied to the dorsum of the thorax with a topical applicator. Treatments were prepared as described in the oral toxicity section above. Individual female flies were held separately in 150-ml plastic cups (Dixie Business, Atlanta, GA) to prevent them from feeding on bait applications applied to other flies. Each cup was covered with a screen cloth lid which was held in place with a rubber band. A 5-cm piece of dental wick moistened in a sugar water solution was placed on top of the screen for each cup. The number of dead flies was evaluated 72 h after application. This study was replicated 5 times for each species.

Statistical analysis. Concentration–mortality regressions for each species by sex were estimated by probit analysis (Finney 1971), using the SAS probit procedure (SAS Institute 1999). Control mortality was corrected using Abbott's formula (Abbott 1925). Differences in toxicity were considered significant when 95% confidence limits (CL) did not overlap.

Results

Oral toxicity. The 24 h oral LC50 estimates for female *B. cucurbitae* and *B. dorsalis* were 113 and 108 mg ai/l, respectively, and not significantly different based on CL overlap (Table 1). Female *B. cucurbitae* were significantly more susceptible than males, but female and male *B. dorsalis* were equally susceptible after the oral route of exposure (Table 1). At the LC90, female and male *B. dorsalis* were significantly more susceptible than female and male *B. cucurbitae*.

Topical toxicity. Female *B. cucurbitae* were significantly less susceptible than female *B. dorsalis* at the LC50 after topical exposure (Table 2). At the LC50, female *B. cucurbitae* were significantly less susceptible to the fipronil-bait mixture after topical exposure compared with females exposed through feeding (Tables 1, 2). However,

Table 1. Feeding toxicity (72 h) of fipronil in Solulys bait.

Sex	Species	No. tested	Slope ± SE	LC50 (95% CI) mg ai/l	LC90 (95% CL) mg ai/l
Female	*B. cucurbitae*	1,050	1.44 ± 0.11	113.4 (96.3 – 134.1)	882.4 (642.7 – 1335.0)
Female	*B. dorsalis*	500	2.81 ± 0.36	108.4 (89.5 – 128.7)	310.0 (243.5 – 447.1)
Male	*B. cucurbitae*	1,050	1.00 ± 0.11	206.1 (159.0 – 283.5)	3,968.0 (2034.0 – 10,878.0)
Male	*B. dorsalis*	500	1.89 ± 0.31	87.9 (63.5 – 111.7)	420.0 (292.9 – 795.8)

Table 2. Topical toxicity (72 h) of fipronil in Solulys bait.

Sex	Species	No. tested	Slope ± SE	LC50 (95% CL) mg ai/l	LC90 (95% CL) mg ai/l
Female	*B. cucurbitae*	200	3.18 ± 0.61	474.0 (381.7 – 570.5)	1,198.0 (908.6 – 2077.0)
Female	*B. dorsalis*	150	5.50 ± 1.64	87.4 (74.0 – 106.0)	141.8 (113.6 – 301.1)

female *B. dorsalis* were equally susceptible to either route of exposure. At LC90 for topical exposure, female *B. dorsalis* were significantly more susceptible than female *B. cucurbitae* (Table 2). At LC90, females of both species were equally susceptible to the fipronil-bait mixture after topical or feeding exposure (Tables 1, 2).

Discussion

Results of this study indicate that there are differences in susceptibility between *B. cucurbitae* and *B. dorsalis* to fipronil, especially at the LC90. *Bactrocera dorsalis* was more susceptible to fipronil than *B. cucurbitae* by oral and topical routes of exposure.

Amulet bait stations used for control of tephritid fruit flies have been developed and tested in the field (Vargas et al. 2005). For *B. dorsalis*, the Amulet stations contain methyl eugenol (Amulet ME), and for *B. cucurbitae* they contain cue lure (Amulet C-L). The concentration of fipronil in these Amulet stations is 5,333 mg ai/l which is substantially higher than the oral LC90 for either of the 2 species evaluated in this study.

Stark et al. (2004) reported spinosad oral LC50 estimates of 4.3 (3.7 - 4.9) and 3.3 (3.1 - 3.6) mg ai/l for female *B. cucurbitae* and *B. dorsalis*, respectively. Thus, spinosad was 26 times more toxic to *B. cucurbitae* and 33 times more toxic to *B. dorsalis* than fipronil.

Potential negative environmental impacts are always a concern when evaluating new insecticides for control of important pest species. Several studies have been published that investigated the potential environmental impact of fipronil. For example, fipronil (Icon 6.2 FSTM) is used as a seed treatment of rice to control rice water weevil, *Lissorhoptrus oryzophilus* Kuschel. Schlenk et al. (2001) found that fipronil was toxic to two crayfish species (*Procambarus clarkia* (Girard) and *P. zonangulus* Hobbs, Jr. and Hobbs III) with LC_{50} estimates of 14 and 19 µg/l, respectively. Schlenk et al. (2001) also conducted an in situ study in culturing ponds containing fipronil and found high levels of mortality in crayfish. A hazard quotient revealed that fipronil in water from Icon-treated rice seed plantings posed a significant risk to crayfish survival.

In contrast to the study by Schlenk et al. (2001), a study by Lahr (1998) indicated that fipronil was one of the least ecologically damaging insecticides to aquatic ecosystems in the Sahel, Africa.

Chandler et al. (2004) studied the effects of fipronil on the estuarine copepod, *Amphiascus tenuiremis* Mielke. Fipronil was highly toxic to *A. tenuiremis* with a 96 h acute LC_{50} of 6.8 µg/l. Additionally, exposure to fipronil delayed female and male development and nearly eliminated reproduction after exposure to 0.42 µg/l.

Stark and Vargas (2005) found that fipronil was very toxic to the water flea, *Daphnia pulex* Leydig, with an LC50 estimate of 16 µg ai/l. However, a hazard assessment

whereby the expected environmental concentration (based on applications of fipronil at concentrations used to control tephritid fruit flies) is compared with the LC50 indicated that fipronil did not pose a hazard to *D. pulex* (Stark and Vargas 2005).

Therefore, it appears that fipronil may be hazardous to some aquatic organisms at environmentally relevant concentrations but not to others. These factors should be taken into consideration if fipronil is to be applied near surface water systems.

In conclusion, fipronil was more toxic to *B. dorsalis* than to *B. cucurbitae* by oral and topical routes of exposure. LC90 estimates were much lower that the 5,333 mg ai/l applied to Amulet Bait Stations for control of *B. cucurbitae* and *B. dorsalis*. Fipronil was much less toxic to these 2 fruit fly species than was spinosad. The results of this study indicate that fipronil is a good choice for control of the *Bactrocera* fruit fly species.

Acknowledgment

The USDA-ARS Hawaii Area-Wide Pest Management Program (SCA # 58-5320-1-498) provided financial support for this work.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Barry, J. D., R. I. Vargas, N. W. Miller and J. G. Morse. 2003. Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera: Tephritidae) in the presence of spinosad bait. J. Econ. Entomol. 96: 1405-1411.

Barry, J. D., S. Polavarapu and L. A. F. Teixeira. 2004. Evaluation of traps and toxicants in an attract-and-kill system for *Rhagoletis mendax* (Diptera: Tephritidae). J. Econ. Entomol. 97: 2006-2014.

Burns, R. E., D. L. Harris, D. S. Moreno and J. E. Eger. 2001. Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. Fla. Entomol. 84: 672-678.

Chandler, G. T., T. L. Cary, D. C. Volz, S. S. Walse, J. L. Ferry and S. L. Klosterhaus. 2004. Fipronil effects on estuarine copepod (*Amphiascus tenuiremis*) development, fertility, and reproduction: a rapid life-cycle assay in 96-well microplate format. Environ. Toxicol. Chem. 23: 117-124.

Chandler, J. 2004. Fruit flies - problems, economic importance and current and proposed control methods. Intern. Pest Cont. 46: 162-166.

Daane, K. M., D. D. Dahlsten and S. H. Dreastadt. 1990. Effects of Mediterranean fruit fly malathion bait spray on the longevity and oviposition of parasitoids of linden and tuliptree aphids (Homoptera: Aphididae). Environ. Entomol. 19: 1130-1134.

Ehler, L. E. and P. C. Endicott. 1984. Effect of malathion bait sprays on biological control of insect pests of olive, citrus, and walnut. Hilgardia 52: 1-47.

Finney, D. J. 1971. Probit analysis, 2nd ed. Cambridge Univ. Press, Cambridge.

Hoelmer, L. A. and D. L. Dahlsten. 1993. Effects of malathion bait spray on *Aleyrodes spiraeoides* and its parasitoids in northern California. Environ. Entomol. 22: 49-56.

Jackson, D. S. and B. G. Lee. 1985. Medfly in California 1980-1982. Bull. Entomol. Soc. Am. 31: 29-37.

Lahr, J. 1998. An ecological assessment of the hazard of eight insecticides used in desert locust control, to invertebrates in temporary ponds in the Sahel. Aquat. Ecol. 32: 153-162.

Peck, S. L. and G. T. McQuate. 2000. Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. J. Econ. Entomol. 93: 280-289.

Purcell, M. F., J. D. Stark and R. H. Messing. 1994. Insecticide effect on thee tephritid fruit flies and associated braconid parasitoids in Hawaii. J. Econ. Entomol. 87: 1455-1462.

Robinson, A. S. and G. H. S. Hooper. 1989. World Crop Pests, Fruit Flies, Their Biology, Natural Enemies and Control. Elsevier Science Publishers, Amsterdam.

Roessler, Y. 1989. Insecticidal bait and cover sprays, Pp. 329-336. *In* Robinson, A.S. and G. Hooper (eds.), Fruit Flies: Their Biology, Natural Enemies and Control, Elsevier, Amsterdam.

SAS Institute. SAS User's Guide: Statistics. 1999. SAS Institute, Cary, NC.

Schlenk, D., D. B. Huggett, J. Allgood, E. Bennett, J. Rimoldi, A. B. Beeler, D. Block, A. W. Holder, R. Hovinga and P. Bedient. 2001. Toxicity of fipronil and its degradation products to *Procambarus* sp.: field and laboratory studies. Arch. Environ. Contam. Toxicol. 41: 325-332.

Stark, J. D., R. I. Vargas and R. K. Thalman. 1990. Azadirachtin: Effects on metamorphosis, longevity, and reproduction of three tephritid fruit fly species. J. Econ. Entomol. 83: 2168-2174.

Stark, J. D., R. I. Vargas and N. Miller. 2004. Toxicity of spinosad in protein bait to three economically important tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). J. Econ. Entomol. 97: 911-915.

Stark, J. D. and R. I. Vargas. 2003. Demographic changes in *Daphnia pulex* (leydig) after exposure to the insecticides spinosad and diazinon. Ecotoxicol. Environ. Saf. 56: 334-338.

Stark, J. D. and R. I. Vargas. 2005. Toxicity and hazard assessment of fipronil to *Daphnia pulex*. Ecotox. Environ. Saf. 62: 11-16.

Steiner, L. F. 1961. The role of attractants in the recent Mediterranean Fruit Fly eradication program in Florida. J. Econ. Entomol. 54: 30-35.

Vargas, R. I., H. Chang and D. L. Williamson. 1983. Evaluation of sugarcane bagasse larval diet for mass production of the Mediterranean fruit fly (Diptera: Tephritidae) in Hawaii. J. Econ. Entomol. 76: 1360-1362.

Vargas, R. I., J. D. Stark, B. Mackey and R. Bull. 2005. Weathering trials of Amulet™, Cue-Lure and Amulet™ methyl eugenol attract and kill stations with male melon flies and oriental fruit flies (Diptera: Tephritidae) in Hawaii. J. Econ. Entomol. 98: 1551-1559.