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Source: Journal of Insect Science, 3(8) : 1-9

Published By: Entomological Society of America

URL: https://doi.org/10.1673/031.003.0801

Michaud JP. 2003. Toxicity of fruit fly baits to beneficial insects in citrus. 9pp. *Journal of Insect Science*, 3:8, Available online: insectscience.org/3.8

Journal
of
Insect
Science

insectscience.org

Toxicity of fruit fly baits to beneficial insects in citrus.

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Received 31 January 2003, Accepted 17 March 2003, Published 20 March 2003

Abstract

Two fruit fly baits, Nu-Lure®/malathion and GF-120 (Spinosad®) were evaluated in the laboratory for non-target impacts on beneficial insects. Nu-Lure/malathion proved attractive and toxic to adults and larvae of the coccinellid species, *Curinus coeruleus* Mulsant, *Cycloneda sanguinea* L. and *Harmonia axyridis* Pallas, a lacewing species, *Chrysoperla rufilabris* Burmeister. The coccinellids *Olla v-nigrum* Mulsant, *Scymnus* sp. and nymphs of the insidious flower bug, *Orius insidiosus* (Say) did not succumb to Nu-Lure baits, even in no-choice situations. Nu-Lure was also attractive and lethal to adults of two aphidophagous flies; *Leucopis* sp. and the syrphid fly *Pseudodorus clavatus* (F.). Both Nu-Lure and GF-120 caused significant mortality to the parasitoid wasps, *Aphytis melinus* De Bach and *Lysiphlebus testaceipes* Cresson, within 24 h of exposure. However, GF-120 caused no significant mortality to any coccinellid in either choice or no-choice situations, despite considerable consumption of baits. Adults of *P. clavatus* tended to avoid GF-120, although mortality was significant in no-choice tests. Although larvae and adults of the lacewing *C. rufilabris* consumed GF-120, mortality was delayed; adults died 48 -96 h post-exposure and those exposed as larvae died two weeks later in the pupal stage. The Nu-Lure bait did not appear palatable to any of the insects, but the high concentration of malathion (195,000 ppm) caused rapid mortality to susceptible insects. Nu-Lure bait without malathion also caused significant mortality to flies and lacewings in cage trials. Although GF-120 bait appeared more benign overall, further research efforts are warranted to increase its selectivity for target fly species and reduce its attractiveness to parasitoids and lacewings. I conclude that the Florida “fly free zone” protocol in its current form is not compatible with an IPM approach to commercial citrus production.

Keywords: *Aphytis melinus, Chrysoperla rufilabris, Curinus coeruleus, Cycloneda sanguinea, Exochomus childreni, Harmonia axyridis, Leucopis* sp., *Lysiphlebus testaceipes, Olla v-nigrum, Orius insidiosus, Pseudodorus clavatus, Scymnus* sp., malathion, non-target effects, Nu-Lure®, Spinosad®.

Introduction

Malathion is a broad-spectrum organophosphate insecticide with a wide range of currently approved uses in agriculture, leading to a total estimated use of more than 16 million pounds per year in the United States (Environmental Protection Agency, 2002). Although the risk posed by malathion to humans and other vertebrates has been extensively studied (Environmental Protection Agency, 2000), the potential impact of large-scale applications of malathion on the structure of insect communities is less well understood. For example, malathion is an active ingredient in bait sprays applied for control or eradication of tephritid fruit flies, e.g. the Mediterranean fruit fly (medfly), *Ceratatis capitata* (Environmental Protection Agency, 1998). During the 1998 medfly eradication program in Florida, malathion was used combined with the corn protein hydrolysate bait, Nu-Lure®. This mixture was applied at a rate of 2.4 fluid ounces malathion and 9.6 fluid ounces Nu-Lure per acre every 7-10 days in regions where Mediterranean fruit fly was detected. In 1998, over 6,285 gallons of ULV malathion (approximately 98% active ingredient) were applied in combination with Nu-Lure over 128 square miles in five Florida counties. Surveys were conducted to detect illnesses in human populations potentially related to these applications (Center for Disease Control, 1999). No information was collected to determine how non-target, insect populations may have been impacted by these eradication efforts. In fact, many beneficial insects, particularly parasitoids, are known to be more susceptible to malathion than fruit flies (Purcell *et al*., 1994).

Another application for malathion-based fruit fly baits is in citrus production in order to comply with the “Caribbean

Fruit Fly-Free Protocol" for fresh fruit export certification in Florida (Florida Division of Plant Industry 2002). This protocol requires the deployment of traps to monitor for the Caribbean fruit fly, *Anastrepha suspensa*, in citrus groves in conjunction with the removal of alternative host plants and ground and aerial applications of Nu-Lure/malathion to prevent infestation. A considerable portion of fresh market citrus acreage in Florida is registered annually in this program by the Florida Division of Plant Industry in order for the fruit to be certified for international export. For example, approximately 184,000 acres of commercial citrus in Florida were registered as 'fly free' in 2001 (Nigg *et al*., 2002). No effort has been made to determine potential side effects of this program on beneficial arthropods in the Florida insect ecosystem, despite evidence of biological control disruption in California resulting from similar spray programs directed against medfly (Troetschler, 1983). The potential for development of fruit fly resistance to malathion in response to broadcast sprays over large areas, such as has been observed for the oriental fruit fly, *Bactrocerca dorsalis* (Hendel) in Taiwan (Hsu and Feng, 2000) is a further impetus for seeking bait sprays with alternative active ingredients.

Dow Agrosciences developed an alternative fruit fly bait, 'GF-120', that utilizes Spinosad as an active ingredient. Initial laboratory tests indicated susceptibility of *A. suspensa* to topical sprays of Spinosad (King and Hennessey, 1996) and field trials in Florida citrus indicated reasonable efficacy of this bait against Medfly and Caribbean fruit fly and relative safety for honey bees (Burns *et al.,* 2001). Spinosad is a combination of spinosyn A and spinosyn B, which are naturally occurring compounds derived from soil-dwelling bacteria. Because the contact toxicity of Spinosad is very low for both vertebrates and invertebrates, and the active ingredient must be consumed in order to cause toxicity, the material is considered acceptable for use in 'organic' agriculture (Organic Materials Review Institute, 2002). Consequently, foliar applications of this material may spare predatory or parasitic insects that do not consume leaves or other plant parts. However, the potential ecological consequences of Spinosad-based fly baits warrant independent scrutiny, since beneficial insects are more likely to consume the material when it is presented in a bait formulation.

Citrus production in Florida relies upon integrated pest management with a large biological control component to maintain populations of many pest insects and mites below economically injurious levels. Because of the paucity of information on the impact of either malathion- or Spinosad-based baits on beneficial insect species in Florida citrus, I assessed the relative attractiveness and toxicity of these baits to a range of beneficial insects representing five different insect orders.

The species selected for these experiments are all of recognized importance in biological control of citrus pests. The chrysopid, coccinellid and syrphid species are important as generalist predators of aphids, psyllids, scales and leafminers (Michaud, 1999; 2000; 2001; 2002; Belliure and Michaud, 2001) and the two parasitoids, for biological control of citrus aphids (Michaud and Browning, 1999) and California red scale (Moreno and Luck, 1992), respectively. A single anthocorid species predatory on thrips was included to represent predatory Hemiptera.

Materials and Methods

Insects

Insect species were selected for testing on the basis of their importance in biological control in citrus, and their availability for collection locally or from commercial sources. The species used included:

Coleoptera: Coccinellidae
Curinus coeruleus Mulsant, *Cycloneda sanguinea* L., *Exochomus childreni* Mulsant, *Harmonia axyridis* Pallas, *Olla v-nigrum* Mulsant, *Scymnus* sp.

Diptera: Chamaemyiidae
Leucopis sp.

Diptera: Syrphidae
Pseudodorus clavatus (F.)

Hemiptera: Anthocoridae
Orius insidiosus (Say)

Hymenoptera: Aphelinidae
Aphytis melinus DeBach;

Hymenoptera: Aphidiidae
Lysiphlebus testaceipes Cresson

Neuroptera: Chrysopidae
Chyrsoperla rufilabris Burmeister

All insects were held on a laboratory bench at a constant temperature of 24 ± 1 °C under fluorescent lighting (L:D 16:8) prior to use in experiments. Colonies of ladybeetles (*C. sanguinea*, *E. childreni*, *H. axyridis*, and *O. v-nigrum*) were established from adults field-collected in Polk County, Florida in May, 2002. The colony of *C. coeruleus* was established using adults field-collected in St. Lucie County, Florida in August, 2001. Adult beetles were maintained in 1 L, wide-mouth mason jars filled with shredded wax paper and covered with muslin. Beetles in jars were fed a combination of bee pollen and frozen eggs of the flour moth *Ephestia kuehniella* Zeller with distilled water available continuously on a cotton wick. Ovipositing females were removed from the jars, isolated in plastic Petri dishes (5.5 cm diameter. x 1.0 cm) and provided with frozen *Ephestia* eggs and water encapsulated in polymer beads (Entomos LLC, www.anbp.org/Entomos.htm) as required. Eggs were harvested daily and held in a Plexiglass incubator until eclosion 3-4 days later (7-8 days for *C. coeruleus* and *E. childreni*). Newly eclosed larvae were reared in Petri dishes (as above) on an *ad libitum* diet of frozen *Ephestia* eggs with water available in polymer beads. Adults of these species were all tested at 2-3 weeks of age; larvae were in the third instar.

Late instar larvae of *Scymnus* sp. were collected from a *Hibiscus* bush infested with *Aphis gossypii* Glover in Polk County in June, 2002 and fed through to pupation on a diet of *A. gossypii* grown on potted cotton seedlings. Adults were tested in the first 24 h following emergence without prior access to food.

Mummies of *L. testaceipes* were collected from colonies of *A. gossypii* on *Hibiscus* sp. in June, 2002 in Polk County, Florida. Mummies were placed in wax paper cups (volume = 480 cm^3) covered with a plastic Petri dish lid (9.5 cm diameter) for emergence. Upon emergence, adult wasps were removed by gentle aspiration through a hole in the cup, sexed, and used in experiments when they were < 24 h old. They had no prior access to food.

Adults of *A. melinus* were obtained by mail order from Biocontrol Network (www.biconet.com/). Wasps were shipped with dilute honey as food, were 24-48 hr old when received, and were used immediately.

Eggs of *O. insidiosus* were obtained by mail order from Entomos (Entomos, LLC) and held in a climate-controlled growth chamber at 24 ± 1 ºC, 60 ± 10% RH, 16:8 L:D until hatching. As the eggs hatched, nymphs were isolated in individual Petri dishes (5.0 cm diameter x 1.0 cm), provisioned *ad libitum* with *Ephestia* eggs and water beads, and used in experiments when they reached the third instar.

Eggs of *C. rufilabris* were obtained by mail order from Beneficial Insectary (www.insectary.com/) and held in a climate-controlled growth chamber at 24 ± 1 ºC, 60 ± 10% RH, 16:8 L:D until hatching. After hatching, larvae were reared individually in plastic Petri dishes (5.5 cm diameter x 1 cm.) on an *ad libitum* diet of *Ephestia* eggs. Larvae were used in experiments in the third instar; adults were tested within 24 h of emergence without prior access to food.

Larvae of *P. clavatus* were collected from ornamental plantings of *Viburnum odoratissimum* in Polk County in June, 2002, where they were feeding on *Aphis spiraecola* Patch. Larvae were placed individually in plastic Petri dishes (5.5 cm diameter x 1 cm ht.) and fed a diet of *A. spiraecola* harvested daily from infested *Viburnum* bushes until they pupated. Adult flies were used in experiments within 24 h of emergence, without prior access to food.

Larvae of *Leucopis* sp. were collected from ornamental plantings of *Viburnum* sp. in Polk County in June, 2002, where they were feeding on *Aphis spiraecola* Patch. Larvae were placed individually in plastic Petri dishes (2.5 cm diameter x 0.8 cm ht.) and fed an *ad libitum* diet of *A. spiraecola* harvested daily from infested *Viburnum* bushes until they pupated. Adult flies were used in experiments within 24 h of emergence without prior access to food.

Chemicals

Malathion (Fyfanon® ULV, Cheminova Inc., http://www.cheminova.us.com) was used at 20% concentration in a corn protein hydrolysate bait, Nu-Lure® (Miller Chemical and Fertilizer Co., www.millerchemical.com). GF-120 was obtained from Dow Agrosciences (www.dowagro.com/) in two forms, with and without Spinosad (0.02%) as the active ingredient.

Experiments

All experiments were performed on a laboratory bench under fluorescent lighting (L:D = 16:8) at a constant temperature of 24 ± 1 °C. Both larvae and adults of *C. sanguinea*, *C. coeruleus*, *H. axyridis*, *O. v-nigrum*, and *C. rufilabris* were tested in choice trials in wax paper cups. Only adult flies and wasps were tested. Bait choice trials were also performed in cages with adults of *C. rufilabris* and *P. clavatus*. Baits were presented in ‘no-choice’ situations to larvae and adults of *C. sanguinea*, *C. coeruleus*, *H. axyridis*, *O. v-nigrum*, and *C. rufilabris* and adults of *P. clavatus*. Care was taken to ensure that equal numbers of males and females were represented in control and treatment groups of all experiments for adult insects that could be sexed (*A. melinus*, *L. testaceipes*, *C. sanguinea*, *C. coeruleus*, *H. axyridis*, and *P. clavatus*). Replication varied among experiments depending on the number of insects available.

Choice Trials - Cups

Experiments were performed with insects (third instar larvae or adults) isolated individually in wax paper cups (accessible volume = 480 cm^3) covered with a plastic Petri dish lid (9.5 cm diameter). Adults of *A. melinus* and *Scymnus* sp., larvae of *E. childreni* and nymphs of *O. insidiosus* were tested in Petri dishes (5.0 cm diameter x 1.0 cm). All insects were presented with a 1 μl droplet of either Nu-Lure bait or GF-120 bait on the lid of the dish, plus a 1 μl droplet of 50% honey solution. Control insects received a 1μl droplet of ‘blank’ bait (either Nu-Lure bait without the malathion or GF-120 bait without the Spinosad). The exceptions were adults of *C. rufilabris* and *P. clavatus*; these received four 1μl droplets each of bait and diluted honey per insect due to their size. Droplets were placed 2 to 3 cm apart on each lid. All predacious larvae, nymphs and coccinellid adults in both treatments received ~ 5.0 mg frozen *Ephestia* eggs placed in the bottom of the cup. A series of between five and ten direct observations were made at intervals of 30 to 60 minutes during the experiments and insect contacts with baits and honey droplets were recorded. Mortality was tallied after 24 h and the number of baits completely consumed was also recorded. The number of insects surviving in control and treatment groups was compared with a Chi-square goodness-of-fit test. Another Chi-square test was performed to compare survival between Nu-lure/malathion and GF-120/Spinosad treatments.

Choice Trials - Cages

To verify that responses to baits measured in the cup choice trials were representative of responses to baits in a larger scale arena, choice bait trials were repeated with adults of *C. rufilabris* and *P. clavatus* in square Plexiglass cages (volume = 0.63 m^3). Cages were prepared with 4 1μl baits and 4 1μl honey droplets per insect, as in the cup trials, control cages receiving blank baits. Groups of 6 -16 newly emerged adults (< 24 h old) were then released in each cage (n = 12 for treatment and control). For adults of *P. clavatus*, bait and diluted honey droplets were applied to Petri dish lids (9.5 cm diameter) placed on the bottom of the cage prior to release of the insects. A series of 8 alternating honey and bait droplets were arranged in a circular pattern on each lid (one lid for each pair of insects), with droplets separated by a distance of approximately 2 cm. For *C. rufilabris* adults, Petri dish lids were taped to one wall of the cage, since previous observations indicated that these insects preferred to feed on vertical, rather than horizontal, surfaces. Mortality and the number of baits completely consumed were both assessed after 24 h. The number of insects surviving was compared across treatment groups with a one-way ANOVA followed by LSD for separation of means (P d” 0.05) (SPSS, 1998).

No-choice Trials

The two parasitoid species appeared almost uniformly susceptible to both baits in the choice trials, but some predatory species seemed unaffected by either bait. To better ascertain the potential toxicity of baits, as well as the acceptability of baits in the absence of alternative food sources, no-choice trials were performed with predators under conditions identical to the choice trials in wax paper cups. Treatment insects were presented with a single 1µl droplet of Nu-Lure or GF-120 bait plus a single 1µl droplet of distilled water applied to the lid of the dish. Control insects received a 1µl droplet of blank bait plus a 1µl droplet of water. Droplets were placed 2 to 3 cm apart on each lid. A series of between five and ten direct observations were made on all insects in all experiments at intervals of 30-60 minutes following initiation of the experiment. Bait consumption and mortality were determined after 24 h, whereupon insects were transferred to plastic Petri dishes (5.5 cm diameter x 1.0 cm) and provisioned *ad libitum* with food and water. Adult *C. rufilabris* and *P. clavatus* were left in the cups, but baited lids were removed and replaced with 4 1µl droplets of diluted honey. Adult *P. clavatus* were held for three days following the no-choice trials, and adult *C. rufilabris* for five days; immature stages were reared through to emergence of the adult. The number of insects surviving in control and treatment groups was compared with a Chi-square goodness-of-fit test. Another Chi-square test was performed to compare survival between Nu-Lure/malathion and GF-120/Spinosad treatments.

Results

Choice Trials – Cups

Adults of *E. childreni* and adults and larvae of *O. v-nigrum* were never observed to approach or consume the Nu-Lure bait, with or without malathion, in the choice trials and did not sustain mortality significantly different from controls (Table 1). Individuals of all other species were observed to taste Nu-Lure/malathion bait on at least one occasion, and these species all sustained significant mortality by the end of the experiment. All observed contacts with Nu-Lure/malathion bait resulted in almost immediate death of the insect. Although all insects were observed to taste GF-120/Spinosad baits on at least one occasion, only the two parasitoid species sustained significant mortality in the choice trials with this bait (Table 1). Comparisons between Nu-Lure/malathion and GF-120 Spinosad treatments revealed no significant differences in survival ($P > 0.05$) for *A. melinus*, *L. testaceipes*, *O. insidiosus*, *Scymnus* sp., *O. v-nigrum* (adults and larvae), and *E. childreni* adults. Significantly more *E. childreni* larvae survived the GF-120/Spinosad (Chi-Square = 5.00; $P < 0.05$), as did adults of *Leucopis* sp. (Chi-Square = 4.90; $P < 0.05$). All other insects survived GF-120/Spinosad exposure better than Nu-Lure/malathion with Chi-square values > 10.00, $P < 0.001$.

No detectable portion of any Nu-Lure bait droplet was consumed by any insect in these trials. Insects died immediately upon contact with Nu-Lure/malathion bait. All species were frequently observed feeding from the honey droplets, apparently in preference to either type of bait. Although consuming the honey preferentially, 65% of *H. axyridis* adults and 100% of *O. v-nigrum* adults also completely consumed the GF-120 control baits, respectively, and 80% and 100% completely consumed the GF-120/Spinosad baits. Larvae of *H. axyridis* and *O. v-nigrum* also found GF-120 to be an acceptable food; 40% and 10% consumed GF-120/control baits and 20% and 15% consumed GF-120/Spinosad baits, respectively. A single adult *C. sanguinea* consumed a GF-120/control bait and another, a GF-120/Spinosad bait. All other insects either avoided GF-120 baits or failed to consume detectable amounts.

Choice Trials – Cages

When adult *P. clavatus* were released in cages with equal amounts of diluted honey and Nu-Lure, mortality was significantly higher than when the GF-120/Spinosad bait was presented, regardless of whether or not the Nu-Lure bait contained malathion (Figure 1), although the addition of malathion more than doubled mortality. There was no detectable consumption of either type of bait in these trials, either with or without its active ingredient, although a large number of honey droplets were completely consumed in all replicates (not quantified).

Cage choice trials with adult *C. rufilabris* produced a

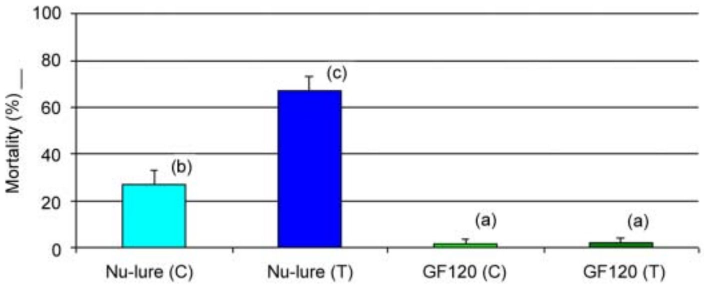

Figure 1. Percentage mortality of adult *Pseudodorus clavatus* after 24 h in cage choice trials with Nu-Lure and GF-120 baits. Insects (n = 12 cages per treatment, 6-16 insects per cage) were presented with 4 1 µl droplets of diluted honey and 4 1 µl droplets of bait per insect on plastic Petri-dish lids on the floor of the cage. Control insects received 'blank' baits without the active ingredient (Nu-Lure = malathion, GF-120 = Spinosad). Data were analyzed by one-way ANOVA followed by LSD (P d” 0.05).

similar pattern of results (Figure 2). Nu-Lure bait produced significantly higher mortality than GF-120 bait regardless of whether or not it contained malathion; Nu-Lure/malathion bait resulted in more than 4 times the mortality of Nu-Lure/control bait. A mean of 38.7 % of GF-120 baits were completely consumed across all trials (n = 24) with many additional baits partially consumed. This compared to no detectable consumption of any Nu-Lure baits.

No-Choice Trials

The no-choice bait trials yielded results that were largely comparable to those obtained in choice trials (Table 2). Both adults and larvae of *O. v-nigrum* survived in the presence of Nu-Lure/malathion as they did in the choice trials. Larvae of *E. childreni* and *C. rufilabris* appeared to increase their acceptance of Nu-Lure/malathion baits in the no-choice situation and died at higher rates than they did in the choice trials. As in choice trials, there was no detectable consumption of any Nu-Lure

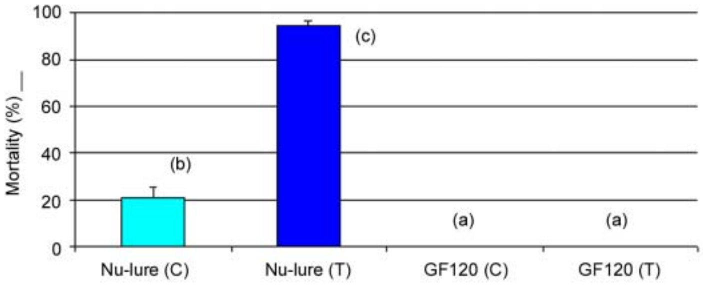

Figure 2. Percentage mortality of adult *Chrysoperla rufilabris* after 24 h in cage choice trials with Nu-Lure and GF-120 baits. Insects (n = 12 cages per treatment, 6-16 insects per cage) were presented with 4 1 µl droplets of diluted honey and 4 1 µl droplets of bait per insect on plastic Petri-dish lids taped to the wall of the cage. Control insects received ‘blank’ baits without the active ingredient (Nu-Lure = malathion, GF-120 = Spinosad). Data were analyzed by one-way ANOVA followed by LSD (P d” 0.05).

Table 1. Percentage mortality of various beneficial insect species presented with two different fruit fly baits. Trials with all insects except *Scymnus* sp., *A. melinus*, and *O. insidiosus* were performed in wax paper cups (surface area = 98.1 cm^2). Trials with the former insects were performed in Petri dishes (surface area = 11.8 cm^2) due to their small size. Alternative food was supplied in the form of 1 µl droplets of diluted honey and, for coccinellids and chrysopid larvae, frozen *Ephestia* eggs. Control insects received ‘blank’ baits (bait formulations without the active ingredient), whereas treatment insects received baits containing the active ingredient (Nu-Lure = malathion, GF-120 = Spinosad). Adult insects were held for 24 h post-treatment; larvae were reared out to adults. Differences in mortality between control and treatment insects were compared by a Chi-square goodness-of-fit test performed on numbers of surviving insects. *(C/T) = n (control group) / n (treatment group)

		% Mortality				% Mortality			
Family / Species	Life stage	Nu-Lure Control	Nu-Lure Treatment	n (C / T)*	*P*	GF-120 Control	GF-120 Treatment	n (C / T)*	*P*
Coccinellidae									
Curinous coeruleus	adults	0.0	90.0	50/50	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	100.0	20/20	< 0.001	0.0	0.0	20/20	ns
Cycloneda sanguinea	adults	25.0	65.0	20/20	< 0.05	5.0	0.0	20/20	ns
	3rd instar larvae	5.0	85.0	20/20	< 0.001	0.0	0.0	20/20	ns
Exochomus childreni	adults	0.0	15.0	20/20	ns	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	50.0	20/20	< 0.05	0.0	0.0	20/20	
Harmonia axyridis	adults	0.0	83.3	18/18	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	100.0	20/20	< 0.001	0.0	0.0	20/20	
Olla v-nigrum	adults	0.0	0.0	20/20	ns	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	0.0	20/20	ns	0.0	0.0	20/20	ns
Scymnus sp.	adults	0.0	20.0	10/10	ns	0.0	0.0	14/13	ns
Chamaemyiidae									
Leucopis sp.	adults	0.0	70.0	10/10	< 0.05	0.0	0.0	10/10	ns
Syrphidae									
Pseudodorus clavatus	adults	18.5	100.0	27/23	< 0.001	4.8	9.5	21/21	ns
Aphelinidae									
Aphytis melinus	adults	0.0	100.0	25/25	< 0.001	8.0	88.0	25/25	< 0.001
Aphidiidae									
Lysiphlebus testaceipes	adults	17.6	94.1	34/34	< 0.001	0.0	80.0	20/20	< 0.001
Anthocoridae									
Orius insidiosus	3rd instar nymphs	0.0	4.0	25/25	ns	4.0	4.0	25/25	ns
Chrysopidae									
Chrysoperla rufilabris	adults	31.3	90.6	32/32	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	0.0	20/20	ns	0.0	0.0	20/20	ns

bait in any trial with any insect. The GF-120/Spinosad bait caused no mortality to any coccinellid species, although a large number of control and Spinosad baits were completely consumed (Table 3).

Significant numbers of *P. clavatus* adults consumed GF-120/Spinosad baits in the no-choice situation (Table 3) and subsequently died (Table 2), five within 24 h and two more the following day. The GF-120/Spinosad bait was apparently avoided when honey was available (Table 1). Adults of *C. rufilabris* consuming the GF-120/Spinosad bait did not begin to die until 48 h or longer following exposure; twelve died on day 3, three on day 4, and one on day five. Nymphs of *O. insidiosus* survived exposure to both Nu-Lure/malathion and GF-120/Spinosad baits as they did in the choice situations.

Discussion

The two baits tested contained active ingredients with markedly different modes of action. Malathion is a contact neurotoxin that inhibits esterases, particularly acetylcholinesterase in the insect nervous system (O'Brien, 1967). GF-120 bait contains an undisclosed concentration of Spinosad, an insect toxin with a less well understood mode of action (Salgado, 1998; Salgado *et al*., 1998), although recent studies suggest that it alters nicotinic and gamma-aminobutryic acid receptor functions (Sparks *et al.*, 2001). The results obtained in this study suggest that GF-120 is, overall, a less toxic bait for beneficial insects compared to Nu-Lure/malathion, despite its apparently higher palatability. The high dosage of malathion in the Nu-Lure bait, a concentration corresponding to 195,000 ppm, may be part of the reason for this difference in toxicity. The results for *P. clavatus* and *C. rufilabris* were consistent when the scale of the presentation arena was increased from 480 cm^3 to 0.63 m^3. This fact suggests that the results cannot simply be explained by random contact with baits as a function of confinement, but were rather a result of their attraction to the bait.

Elzen et al. (1998) showed found that malathion was the most toxic of the ten insecticides they tested against two hemipterans, one coccinellid and one lacewing, whereas spinosad was the least toxic. Similarly, Elzen (2001) showed that *O. insidiosus* was especially sensitive to malathion. The failure of *O. insidiosus* to sustain significant mortality in trials with Nu-Lure/malathion baits thus strongly indicates a lack of

Table 2. Percentage mortality of beneficial insect species presented with two different fruit fly baits in a no-choice situation. Trials were performed in wax paper cups (surface area = 98.1 cm^2) except for larvae of *E. childreni* that were tested in Petri dishes (5.0 cm diameter). Baits were presented as single 1 µl droplets on Petri dish lids accompanied by a 1 µl droplet of distilled water. Control insects received the 'blank' bait (without the active ingredient), while treatment insects received baits containing either malathion (Nu-Lure) or Spinosad (GF-120). Immature stages were reared through to emergence of adults; adult *P. clavatus* were held for 3 days post-treatment and adult *C. rufilabris* for 5 days. Differences in mortality between control and treatment insects were compared by a Chi-square goodness-of-fit test performed on numbers of surviving insects. *(C/T) = n (control group) / n (treatment group)

		% Mortality				% Mortality			
Family / Species	Life Stage	Nu-Lure Control	Nu-Lure Treatment	n (C / T)*	*P*	GF-120 Control	GF-120 Treatment	n (C / T)*	*P*
Coccinellidae									
Curinous coeruleus	adults	0.0	75.0	20/20	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	5.0	50.0	20/20	< 0.001	5.0	10.0	20/20	ns
Cycloneda sanguinea	adults	0.0	80.0	20/20	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	35.0	20/20	ns	0.0	5.0	20/20	ns
Exochomus childreni	adults	0.0	20.0	20/20	ns	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	75.0	20/20	< 0.001	0.0	0.0	20/20	ns
Harmonia axyridis	adults	0.0	100.0	20/20	< 0.001	0.0	0.0	20/20	ns
	3rd instar larvae	0.0	100.0	20/20	< 0.001	0.0	5.0	20/20	ns
Olla v-nigrum	adults	0.0	15.0	20/20	ns	0.0	0.0	20/20	ns
	3rd instar larvae	10.0	5.0	20/20	ns	0.0	0.0	20/20	ns
Scymnus sp.	adults	0.0	10.0	10/10	ns	0.0	0.0	20/20	ns
Syrphidae									
Pseudodorus clavatus	adults	~	~	~	~	0.0	70.0	10/10	< 0.001
Anthocoridae									
Orius insidiosus	3rd instar nymphs	4.0	16.0	25/25	ns	16.0	34.8	25/23	ns
Chrysopidae									
Chrysoperla rufilabris	adults	0.0	100.0	20/20	< 0.001	10.0	80.0	20/20	< 0.001
	3rd instar larvae	0.0	55.0	20/20	< 0.05	0.0	30.0	20/20	< 0.001

attraction to the bait. The observation that Nu-Lure appeared less palatable than GF-120 to many insects in this study, even without the active ingredient, supports the contention of McQuate *et al.* (1999) that replacement of malathion with alternative active ingredients, such as phloxine B-protein, may have potential for improving the environmental safety of the Nu-Lure bait. However, the significant mortality caused by the Nu-Lure/control bait in the cage choice trials with both *P. clavatus* and *C. rufilabris* raises questions regarding the potential toxicity of other, unidentified, ingredients in the formulation.

Ludwig and Oetting (2001) obtained ambiguous results with their two tests of Spinosad for compatibility with *O. insidiosus* for control of thrips on greenhouse chrysanthemums. Miles and Dutton (2000) reported Spinosad as highly toxic to parasitic Hymenoptera in greenhouses, but concluded it was compatible with *O. insidiosus*, *C. rufilabris* and the coccinellids *Hippodamia convergens* and *Coccinella septempunctata*. Tillman and Mulroney (2000) observed toxicity of Spinosad to three parasitoid species in cotton, *Bracon mellitor*, *Cardiochiles nigriceps* and *Cotesia marginiventris* although the coccinellids *Coleomegilla maculata* and *H. convergens* were unaffected. Similarly, Mason *et al.* (2002) demonstrated toxicity of Spinosad to *Trichogramma inyoense*, an egg parasitoid of *Mamestra configurata*. These results parallel our findings that GF-120/Spinosad bait is highly attractive and lethal to parasitoid wasps, in this case *A. melinus* and *L. testaceipes*. Our results also concur in indicating that GF-120/Spinosad bait is quite safe for coccinellids and for *O. insidiosis*, even though many of the former species readily consume it. However, previous studies suggesting the safety of Spinosad to eggs and pupae of chrysopids (Medina *et al.,* 2001) employed topical applications and did not assay consumption by active life stages. Our results suggested that GF-120/Spinosad is both attractive and toxic to *C. rufilabris*, particularly in the adult stage, with mortality resulting several days later. This delayed toxicity accounts for the lack of mortality observed in the choice trials where insects were held only for 24 h post treatment. For example, a significant amount of GF-120/Spinosad was consumed by *C. rufilabris* adults in the cage choice trials and many of these insects would have likely have died in the following days. Future studies of the toxicity of Spinosad should, therefore, hold insects for at least three days or longer post treatment.

Table 3. Percentage of GF-120 control and treatment baits completely consumed by insects in no-choice situations. Larvae were tested in the third instar. No differences in consumption between control and treatment bait were significant when a Chi-square Goodness-of-fit test was performed on the whole numbers (P > 0.05 in all cases). Note: A zero value does not exclude partial consumption of baits that was not quantifiable.

Species		GF-120 Control	GF-120 Treatment
Coccinellidae			
Curinus coeruleus			
	adults	20	15
	larvae	10	30
Cycloneda sanguinea			
	adults	70	60
	larvae	15	15
Exochomus childreni			
	adults	40	20
	larvae	0	0
Harmonia axyridis			
	adults	95	90
	larvae	90	95
Olla v-nigrum			
	adults	100	100
	larvae	100	100
Syrphidae			
Pseudodorus clavatus			
	adults	60	50
Chrysopidae			
Chrysoperla rufilabris			
	adults	80	60
	larvae	50	55

This work is not alone in illustrating potential negative ecological side effects of Nu-Lure fly bait applications, although it is unique in testing a wide range of insect species. Twenty years ago, Troetschler (1983) demonstrated that various polyphagous predators were attracted to, and killed by, malathion-based bait sprays directed against the Mediterranean fruit fly in California. Daane *et al.* (1990) suggested that malathion-based bait sprays for the Mediterranean fruit fly could disrupt biological control of aphids through non-target impacts on aphid parasitoids. Peck and McQuate (2000) tested malathion- and Spinosad-based bait sprays against the Mediterranean fruit fly in Hawaiian coffee plantations and recommended preferential use of the latter on the grounds of better environmental safety, despite the higher efficacy and longer field persistence of malathion. Although disruptions of biological control resulting from broadcast applications of Nu-Lure against Caribbean fruit fly in Florida citrus have not been reported, it seems likely that appropriate observations have not been collected, or that a possible correlation with pest outbreaks has not been made. Ehler and Endicott (1984) observed outbreaks of scale insects in California citrus following repeated application of malathion-based bait sprays for Mediterranean fruit fly, although these effects were not noted until five months to one year following cessation of the sprays. In the light of the results presented here, and these earlier studies, greater scrutiny should be directed toward the potential negative ecological impacts of the “fly free zone” protocol in Florida citrus.

Acknowledgements

I wish to thank Dr. H. Nigg for providing the fly baits and reviewing the MS, Dr. S. Simpson for insights into the “fly-free zone” protocol, and Ms. L. Tretyak for technical support in these experiments. This work was supported by grants from the Environmental Protection Agency, region 4, and the Florida Citrus Producers Research Advisory Council.

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