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The effects of spinosad on Culex quinquefasciatus (Diptera: Culicidae) and non-target insect species

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THE EFFECTS OF SPINOSAD ON *CULEX QUINQUEFASCIATUS* (DIPTERA: CULICIDAE) AND NON-TARGET INSECT SPECIES

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"Anyone who thinks they are too small to make a difference has never tried to fall asleep with a mosquito in the room."

~Christie Todd Whitman, Former New Jersey Governor and EPA Administrator

"I have the attention span of a mosquito from multitasking and all the things that have affected my poor little brain."

~Ian Somerhalder, American Actor and Model

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ABSTRACT

Spinosad is a relatively new insecticide with a unique mode of action that is being
evaluated for control of larval mosquitoes. Whereas a number of toxicological studies have
measured effects of spinosad on various mammals, fish, birds, and terrestrial arthropods, fewer
studies have been conducted on the effects of spinosad on non-target aquatic insect species.
Such studies are important as these species might be found in the same environments as
mosquito larvae targeted for control. A neighborhood pond was surveyed to find a representative
species of mosquito as well as other common aquatic insects with which to examine
susceptibility to spinosad and non-target effects. The mosquito species chosen was *Culex
quinquefasciatus* and the most common non-target taxa were immature stages of a mayfly
(*Caenis* spp., Ephemeroptera: Caenidae), a damselfly (*Ischnura* spp., Odonata: Coenagrionidae)
and a dragonfly (*Pachydiplax longipennis*, Odonata: Libellulidae). Bioassays of mosquitoes
from a reference susceptible strain (Sebring-S) and field-collections of *C. quinquefasciatus* were
used to determine susceptibility to spinosad. In addition, susceptibility was examined in non-
target taxa using spinosad concentrations corresponding to the LC_{50} of a field-collected
mosquitoes (0.031 ppm) and the labeled rate (1.6 ppm) of Natular[®], an EC formulation of
spinosad. Susceptibility to spinosad did not differ between Sebring-S and field-collected
mosquitoes. However, there was a marked difference in susceptibility among non-target taxa.
Susceptibility was greatest in *Caenis* spp., followed by *Ischnura* spp., then *P. longipennis*.
Results from this study will allow better future management strategies for the use of spinosad as
a mosquito larvicidal agent.

INTRODUCTION

Mosquitoes include some of the most economically and medically important insect species. They are ubiquitous, and capable of carrying and transmitting a spectrum of animal and human diseases, often rapidly across entire continents (Snow et al., 1999; Roth et al., 2010; Weaver et al., 2010). These diseases cause hundreds of millions of clinical cases and millions of deaths annually (Snow et al., 1999; Roth et al., 2010), and stunt both population and economic growth in entire regions (Utzinger et al., 2002; Killeen et al., 2004). For malaria alone, an estimated 225 million clinical cases of infection and 781,000 deaths occurred globally in 2009, and 655,000 deaths in 2010, most of these occurring in sub-Saharan Africa (WHO, 2010; 2011).

Mosquitoes in the genus *Culex* are vectors of a number of debilitating diseases in humans and animals. Among them, the Southern house mosquito, *Culex quinquefasciatus*, is the most prevalent vector of urban and lymphatic filariasis in southern Asia and South America (Regis et al., 2000; Triteeraprapab et al., 2000), which infects over 100 million people worldwide (Myung et al., 1998; WHO, 2009), and is still endemic in at least 83 countries (WHO, 2009). In addition, *C. quinquefasciatus* is one of several prevalent vectors in the *C. pipiens* complex of West Nile virus in North America, which was introduced in New York City in 1999 and spread rapidly to the west coasts of both the U.S. (Goldberg et al., 2010; Weaver & Reisen, 2010) and Canada (Roth et al., 2010). As of 2011, approximately 25,000 clinical infections and over 1,200 deaths have been reported (Murray et al., 2010; CDC, 2011; 2012). Whether or not *C. quinquefasciatus* is capable of transmitting dengue fever has also been debated. However, previous studies suggest that, while it may be possible in lab settings, transmission is unlikely to occur in nature (Rosen et al., 1985; Huang et al., 1992; Vazeille-Falcoz et al., 1999). Historically, *C. quinquefasciatus* has also been a major vector of St. Louis Encephalitis in Central and North

America (Day, 2001), and may have been partially responsible for a sudden outbreak of the disease in the U.S. in 1975 with nearly 2,000 clinical cases (Creech, 1977; CDC, 2009). Finally, *Culex* spp. is a nuisance mosquito and is a predominant target of urban mosquito abatement efforts.

Chemical insecticides are the cornerstone of efforts to manage populations of mosquitoes (Hemingway & Ranson, 2000). Although chemicals have been used successfully as components of management strategies, insecticide susceptibility is best viewed as a limited resource (Hemingway & Ranson, 2000). Thus, all insecticides, regardless of mode of action, will inevitably select for resistance in target species, and novel insecticides must be found to replace them. In addition, unintentional damage to non-pest species that results from insecticide application (non-target effects) limits their utility.

The insecticide, DDT, was among the first used to control arthropod vectors of human diseases, and was originally sprayed to combat malaria and typhus during World War II. By the end of the war, DDT had been used to control mosquitoes that vector malarial pathogens after it had been proven effective in the European theater (CDC, 2010). Largely through applications of DDT, incidence of malaria fell dramatically around the world, and malaria was considered eradicated from the continental U.S. by 1951 (CDC, 2010). In addition, malarial infections were greatly reduced in other regions for the following decades, such as the Philippines (where incidences fell from 68% to as low as 6% in some populations) (Stapleton, 2004), and Ceylon (modern-day Sri Lanka), in which mortality fell from 1,180 cases per million to roughly 250 cases per million from 1941 to 1950 (Gray, 1974).

Largely as a result of environmental contamination and insecticide resistance to DDT, other insecticides such as pyrethroids, carbamates, and organophosphates (OPs) were used for

control of disease vectors (Gericke et al., 2002). Resistance to DDT in mosquitoes was first reported in 1947 in *Anopheles taeniorhynchus* in Florida after only four years of use in that region (Brown, 1986; Chareonviriyapap et al., 1999). By 1960, over 130 different insect species had developed resistance to DDT (Stapleton, 2004), and by 1992, over 100 mosquito species showed resistance (WHO, 1992; Hemingway & Ranson, 2000). The first reports of resistance to DDT in *C. quinquefasciatus* were along the African Coast (Cote d'Ivoire and Burkina Faso) (Adam et al., 1958 as cited in Chandre et al., 1997), and by 1977, DDT resistance was reported for most geographic regions (WHO, 1976; 1980). In addition, by the 1970's, environmental contamination with DDT was widespread (Miskus et al., 1964; Cottam, 1965; Johnson et al., 1971; Stapleton, 2004), at which time OP compounds became predominant insecticides for control of *C. quinquefasciatus* and other pest species (WHO, 1976; 1980).

The OPs were first developed in the 1930's by Dr. Gerhard Schrader of the Bayer Corporation in Germany, but they didn't become available for use as insecticides until 1941 (Busby & Eckstein, 2009). Resistance to OPs in populations of *C. quinquefasciatus* were first reported in 1963 in western Africa (Chandre et al., 1997). By 1977, there had been an increase in the numbers of reports of resistance to numerous OPs in *C. quinquefasciatus* throughout Africa and Asia (WHO, 1976; Chandre et al., 1997) and in regions of the U.S. (Georghiou et al., 1975). By 1987, OP resistance had become prevalent in most major geographic regions. In addition, effects of OPs on non-targets became more recognized within this time, with special concern for its effects on birds (White, 1979; Stone et al., 1984; Hunt et al., 1991; Flieschli et al., 2004) and fish (White, 1979; Khan & Law, 2005; Shoaib et al., 2012). Within this time, OPs were replaced by pyrethroid insecticides, which then became widely used to manage medically important pests (WHO, 1980; 1986).

Pyrethroids are among the most successful classes of insecticidal chemicals. These insecticides, which are synthetic analogs of natural insecticides (pyrethrins) produced in *Chrysanthemum cinerariafolium*, were pioneered by Michael Elliot of the Rothamsted Experimental Research Station throughout the 1960's and 70's (Housset & Dickman, 2009), and were used on mosquitoes and other medically important species throughout the 1980's (WHO, 1980; 1986; 1992). The first reported resistance to pyrethroids in *C. quinquefasciatus* resulted from artificial selection in a laboratory in 1978 (Priester & Georghiou, 1978), and then appeared in wild populations of these mosquitoes in West Africa in 1986 (WHO, 1986; Magnin et al. 1988; Chandre et al., 1998). Since that time, resistance has increased dramatically in these areas (WHO, 1980; 1986; 1992), with resistance reaching levels as high as 82-fold in populations of *C. quinquefasciatus* in Africa in the late 90's (Chandre et al., 1998). Further resistance has been reported in regions of Thailand and in the southern U.S. (Liu et al., 2004; 2009; Thanispong et al., 2008), with resistance to pyrethroids reaching as high as 1,400-fold in some cases (Liu et al., 2009). Despite widespread resistance to both OPs and pyrethroids, members from both classes of insecticides continue to be used for control of adult mosquitoes, largely because few effective alternatives are available.

Spinosad is a new insecticide that shows promise as a mosquito control agent. It is an insecticide derived from *Saccharopolyspora spinosa*, an actinomycete discovered in an abandoned sugar rum mill in the Caribbean by a vacationing chemist in 1982 (Thompson et al., 2000). Four years later, scientists working for Eli Lilly (now a part of Dow Elanco) determined the insecticidal agent to be a mixture of fermentation products (spinosyns A and D) produced by *S. spinosa* (Thompson et al., 2000).

One of the promising attributes of spinosad is that its toxicity is mediated via a mode of action that differs from that of OPs and pyrethroids. Insects ingesting spinosad experience paralysis caused by the rapid excitation of the nervous system through binding to the nicotinic acetylcholine and/or GABA receptors (Salgado, 1998). This action usually causes death in arthropods within 48- to 78-hours after ingestion (Salgado et al., 1998; Thompson et al., 2000). In addition, the fact that it must be ingested increases its selectivity to phytophagous species, especially crop pests.

In its fifteen years of use, resistance to spinosad has been limited in part because of its unique mode of action. Thus, no cross-resistance to spinosad has been measured in mosquitoes that are resistant to pyrethroids or OPs (Darriet et al., 2005). One notable exception was found in two field populations of the diamondback moth (*Plutella xylostella*) in Hawaii and Thailand that showed high (over 100-fold) resistance to spinosad (Zhao et al., 2002). In addition, populations of the western flower thrips, *Frankliniella occidentalis*, collected in southeastern Spain, are up to 46,000-fold resistant to spinosad (Bielza et al., 2007). Finally, populations of the common house fly, *Musca domestica*, were selected in a laboratory with spinosad and developed >150-fold resistance within 10 generations (Shono & Scott, 2003).

Spinosad is highly toxic to a broad array of target pests. This insecticide was first registered in 1997 for use against lepidopteran pests of cotton (Thompson et al., 2000; Legocki et al., 2010), and has since been registered for other lepidopteran pests on other crops, as well as pests in the Orders Diptera (such as fruit flies and mosquitoes), Thysanoptera, Isoptera and Coleoptera (Thompson et al., 2000; Legocki et al., 2010). It is currently registered in over sixty countries and is applied to over 200 crop species, with 180 in the U.S. alone (Legocki et al., 2010). It is also toxic to some Orthoptera, Hymenoptera and Dermaptera (Legocki et al., 2010),

and recently discovered effects against Phthiraptera and Siphonaptera has led to use of spinosad in veterinary medicine (Snyder et al., 2007; Legocki et al., 2010).

The toxicity of spinosad to non-target organisms is relatively low. Spinosad is relatively non-toxic to mammals ($LD_{50} > 5000$ mg/kg), slightly toxic to birds, and slightly-to-moderately toxic to fish. It is highly toxic to honeybees (0.5 ug/g) and parasitic Hymenoptera. However, spinosad degrades rapidly, minimizing potential exposure (USEPA, 1999; Medina et al., 2008). In addition, spinosad has a favorable environmental profile: it is not known to leach, bioaccumulate, or volatilize (Thompson et al., 2000; West et al., 2000; Williams et al., 2003), and also has no known carcinogenic, teratogenic, or mutagenic effects on vertebrates (Legocki et al., 2010). As a result of its limited non-target and chronic effects, in 1993, spinosad was labeled as a "reduced risk" compound by the EPA, and in 1999, Dow AgroSciences received the Presidential Green Chemistry Award for its development.

Spinosad shows promise as a control agent for medically important species. When mosquitoes began to develop resistance to the previous generation of insecticides, mosquito abatement and control agencies began to search for new insecticides with new modes of action (Darriet & Corbel, 2006; Perez et al., 2007). Spinosad was first registered for use against mosquitoes in the U.S. in 2008 under the trade name Natular[®] (Ravichandran, 2011). Ironically, while it is only until recently that spinosad has been used against mosquitoes, its actual insecticidal properties were first detected in a mosquito bioassay in the 1980's (Thompson et al., 2000; Bond et al., 2004). The first primary applications for mosquitoes in the U.S. were against *Aedes albopictus* and *Ae. aegypti* alongside applications against other medically important pests (Legocki et al., 2010). Currently, spinosad is being used (or evaluated for use) in mosquito

control programs both in the U.S. and in a number of foreign countries (Liu et al., 2004; Darriet & Corbel, 2006; Perez et al., 2007; Legocki et al., 2010).

Whereas toxic effects of spinosad on terrestrial arthropods and aquatic vertebrates (e.g., fish, reptiles, water fowl, mammals, etc.) have been well-documented (Eger Jr. et al., 1998; Thompson et al., 2000; Cisneros et al., 2002; Williams et al., 2003), its effects on non-target arthropods in aqueous environments have been less well studied (Dow AgroSciences, 1998, 2002; Stark & Vargas, 2003; Bond et al., 2004; Duchet et al., 2008; Duchet et al., 2010a, 2010b). The potential effects of insecticides on non-targets in aquatic environments can vary widely due to a large number of factors. More predictable effects can include mortality due to insecticide sites of action co-occurring across target (i.e., pest) and non-target species. For example, pyrethroids affect the voltage-sensitive Na+ channels in both insects and mammalian species (Wolansky & Harrill, 2008). In addition, juvenile hormone mimics, such as S-methoprene affect development in crustaceans as well as in insects (Mortimer & Chapman, 1995; Verslycke et al., 2007).

In addition, there are also less predictable effects that can occur in non-targets upon insecticide exposure. A noteworthy case is exposure of frogs in Minnesota to S-methoprene, which affected early development and caused growth of additional appendages and malformed limbs (Cohen, Jr., 2001; Degitz et al., 2003). More recently, neonicotinoid insecticides have been implicated as causal agents in extreme reductions in honey bee and bumble bee populations (Yang et al., 2008; Marzaro et al., 2011; Whitehorn et al., 2012). The difficulty in predicting such non-target effects can be further exacerbated by physical differences (e.g., pH, salinity, temperature, UV exposure, absence or presence of sediments or organic material) between aquatic and terrestrial environments that may affect toxicity and persistence of insecticides in the

environment (Maund et al., 2002; Nevskaia et al., 2004). In addition, fundamental biological processes (e.g., osmoregulation) differ between terrestrial and aquatic species (Siegfried, 1993). Finally, ecological effects, such as bio-accumulation within food webs, are difficult to predict. For example, exposure to DDT applied to Clear Lake, CA at approximately 0.02 ppm to control gnats, in 1949, 1954, and 1957 led to a dramatic decrease in populations of the western grebe. A local population of 1000 breeding pairs failed to produce a single hatchling until 1962 due to DDT poisoning from contaminated fish and other prey (Boellstorff et al., 1985; Cottam, 1965). Tests later showed that plankton (taxa not named) had bioaccumulated up to 5.3 ppm DDT (a 265-fold increase over the maximum amount applied), and the visceral fat of frogs and carp were found to have between 5 to 40 ppm (a 2000-fold increase) (Cottam, 1965). Up to 1,600 ppm DDT were found in some grebes (an 80,000-fold increase), and concentrations were even higher in some species of fish (such as bullheads), in which some specimens had up to 2,700 ppm DDT in their tissue, an increase of 135,000-fold (Cottam, 1965).

Often, potential non-target effects may be detected using a representative resident (i.e., a bioindicator) of a given environment. For example, many studies have used a wide variety of odonates (i.e., dragonflies and damselflies) as bioindicators of heavy metal contamination because of their tendency to sequester toxins that cause a variety of physiological effects., including cadmium, cobalt-60, fluorides and lead (Wixson & Clark, 1967; Murihead-Thompson, 1971; Dewey, 1973; Mathis et al., 1977; Meyer et al., 1986; Westfall Jr. & May, 1996; Needham et al., 2000). The most common sublethal effects, which might be observed in response to a large variety of pollutants, include abnormalities in body size and weight (Palli & Markobatova, 1963 as cited in Westfall Jr. & May, 1996), changes in molting frequency and other developmental effects (Rupperecht, 1975, Mielewczyk, 1977, Subramanian & Varadaraj, 1993;

Westfall Jr., & May, 1996). In addition, toxicological responses of mayflies (order

Ephemeroptera) have been studied extensively. In general, most ephemeropterans are sensitive

indicators of polluted waters, especially poorly oxygenated environments (Deepa, 2012;

Menetrey, 2008). Their fecundity is especially dependent on environmental conditions (Soldan,

1981, as cited by Landa & Soldan, 1995), and because of the short duration of the adult stage,

mobility in populations is low. As such, larval Ephemeroptera are often considered to be

accurate indicators of conditions in local environments (Sweeney & Funk, 1991, as cited by

Landa & Soldan, 1995), and are used as key bioindicators (Deepa, 2012; Fialkowski et al., 2003;

Landa & Soldan, 1995; Menetrey et al., 2008) or components of models used to assess the

overall health of the ecosystem (Deepa, 2012). Previous studies with insecticides and mayflies

have shown mortality and reduced growth in the wild caused by exposure to OPs and

carbamates, as well as fungicides (Hatakeyama et al., 1997; Peterson et al., 2001). Furthermore,

other studies have demonstrated non-lethal effects, such as disruption in osmoregulation

(pyrethroids) (Tang & Siegfried, 1995), drifting of populations downstream (rotenone), and the

possible migration of entire populations away from sites exposed to insecticides (rotenone)

(Arnekleiv et al., 2001).

Whereas research on aquatic invertebrates has been limited, spinosad has been shown to

be toxic to a number of aquatic species, such as water fleas, oysters, and grass shrimp (Dow

AgroSciences, 1998, 2002; Thompson et al., 2000; Bond et al., 2004; Duchet et al., 2008, Duchet

et al., 2010a, 2010b). Given physical and biological differences between terrestrial and aquatic

environments, and the fact that the active components of spinosad are somewhat water-soluble

(235 ppm and 0.332 ppm for spinosyns A and D, respectively, at 25°C, pH 7), the environmental

and toxicological profiles of spinosad could conceivably differ between these two environments. This has not received extensive study.

The objective of my research was to evaluate the efficacy of spinosad against target and non-target species. The toxicity of spinosad against a target species (larval *C. quinquefasciatus*) was determined with a static bioassay and mosquitoes from a reference susceptible strain and field collections. As part of the objective, I surveyed the collection site throughout several seasons to identify and catalogue potential non-target species. Finally, I evaluated the toxicity of spinosad against representative non-target species. Results from this study will allow for safer use of spinosad in mosquito control program

MATERIALS AND METHODS

Chemicals

Spinosad (a mixture of 50–95% (2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR)-2-(6-deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a, 16b-hexadecahydro-14-methyl-1H-as-indaceno[3,2-d]oxacyclododecine-7,15-dione
and 5–50% (2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bS)-2-(6-deoxy-2,3,4-tri-O-methyl-α-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-β-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1H-as-indaceno[3,2-d]oxacyclododecine-7,15-dione) (Natular® EC Formulation) was provided by East Baton Rouge Mosquito and Rodent Control (EBRMARC). A stock solution (10 ppm) was prepared using double-distilled water (ddH$_2$O) and an amber bottle, and stored at room temperature until use.

Field Site

Wild populations of mosquitoes were collected for study at a field site located at Eliza Beaumont Lane, Baton Rouge, LA. (30°25'39.91"N, 91°9'15.43"W). The site is a small pond (Fig. 1-3) aerated by a fountain (Fig. 4) and located in an isolated residential area. It is surrounded and partially covered by oak trees, and has a bridge running to a small island in the center of the pond. The shoreline is populated with emergent vegetation and the benthos is composed of mud that is covered in areas by dead leaves from trees overlooking the pond. The depth varies depending on distance from the shoreline, but does not exceed five feet.
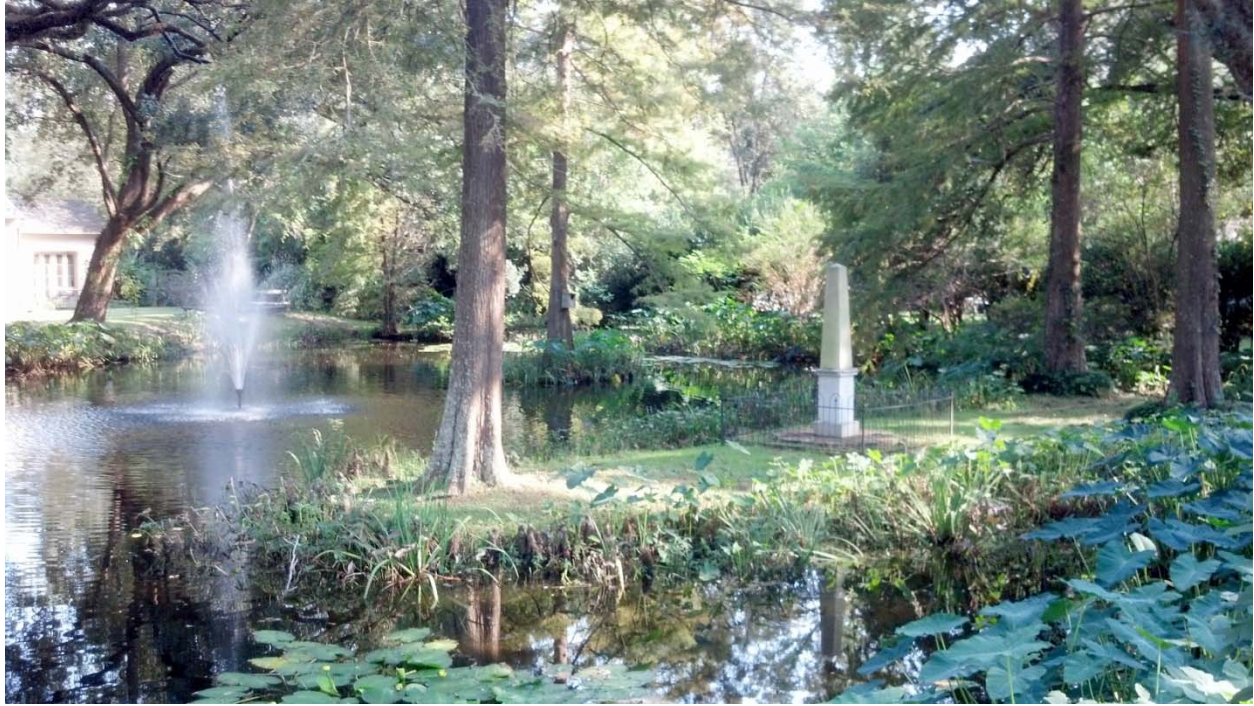
Fig. 1: Survey site at Eliza Beaumont Ln.

Fig. 2: View of the emergent vegetation on the shoreline of the survey site.

Fig. 3: Additional shoreline of the survey site, partially showing the benthos at the shoreline.

Fig. 4: View of the fountain used to aerate the pond water.

Survey of Mosquito Species

Mosquito species at the field site were surveyed using miniature CDC blacklight/CO_2 traps (Sudia & Chamberlain, 1962), which used a single fan powered by a 6-volt electric motor and a single 4-watt fluorescent F4T5 black-light bulb in each. The traps used CO_2 (in the form of dry ice) and a black light as attractants, and were set up and left overnight. The following mornings, captured mosquitoes were transported in collection cups, placed in a freezer at the LSU Entomology Mosquito Research Laboratory for 24 hours, and then transported to EBRMARC using a styrofoam cooler loaded with ice. All mosquitoes were identified with assistance from personnel at EBRMARC. Collection dates were June, 8th, 12th, 16th and 22nd, 2011. Voucher specimens were not retrieved because the freezer in which the specimens were contained in at EBRMARC were destroyed when the freezer failed.

Survey of Insect Species

Field populations of *C. quinquefasciatus* were obtained from the field site using oviposition traps baited with fish emulsion (Alaska Brand Fish Fertilizer, 5-1-1 ratio; 0.14L/Gal water) as an attractant (R. Vaeth, personal communication, 2011). Egg rafts were collected using a section of small wire mesh (0.125 mm aperture) and transported to the LSU Entomology Mosquito Research Lab. Larvae were reared in metal pans filled with double-distilled water (ddH$_2$O) and fed liver powder (provided by MP Biomedicals, LLC., Santa Ana, CA.) until they reached the 3rd instar, at which point they were moved into the mosquito bioassays.

Non-target insects were collected from the study site using a D-frame aquatic sweep net (1.2 mm aperture), a plastic mosquito dip (max volume 350 ml), or a minnow-sieve (1.5 mm aperture). Insects were collected continuously between the times of 9:00 am and 1:30 pm and

were then transported in specimen cups of pond water back to the LSU Entomology Mosquito Research Lab where they were preserved in 90% ethyl alcohol and classified to family and, if possible, genus and species, using a dichotomous key. Specimens along with pond water were then passed through a series of sieves (No. 50, 120, and 270 aperture sizes, or 0.297mm, 0.125mm, and 0.053mm, respectively). The three most abundant non-target species were then used for the non-target bioassays (described below). Collection dates in 2011 were: May 24th, 27th, 30th, June 1st and 3rd, 7th, 9th and 22nd, November 5th, and December 7th. During 2012, collections were made on: February 6th, March 5th, May 19th, May 21st, June 27th, and July 18th. Voucher specimens were turned into the Louisiana State University Arthropod Museum.

Reference Susceptible Strain

A reference, susceptible strain of *C. quinquefasciatus* (SEBRING-S) was obtained from Mosquito Abatement in Harris Country, Texas. The United States Department of Agriculture Agricultural Research Station in Gainesville, FL originally founded the colony from mosquitoes collected in 1988 in Sebring, FL (Sbrana et al., 2005; Johnsen, 2007).

Bioassays

Susceptibilities of mosquito larvae and non-targets were determined using a static system and aqueous solutions of spinosad in Pyrex® petri dishes (No. 3140-100; 100mm x 50mm). Prior to assays, dishes were washed with soap and water, then rinsed with 0.1 M NaOH, rinsed again with water, followed by bulk acetone, rinsed with water again, and then baked in a lab oven at 180°F for 24 hours. A stock solution of spinosad (10 ppm) was prepared by dilution with ddH$_2$O, and stored in the dark at room temperature until use. It was then diluted further to create a 0.1 ppm working solution that was diluted immediately prior to use in bioassays.

Twenty, 3rd instar mosquito larvae from field collections or the reference SEBRING-S strain were introduced into Pyrex® dishes containing 100 mL of insecticide using a small section of wire mesh, and containers were placed in an incubator at a constant temperature (27°C) and photoperiod (14:10-hour L:D). Dishes containing mosquitoes and 100 mL ddH$_2$O were used as the control. Mortality was monitored at 72 hours after initial exposure, and was defined as lack of movement in response to continued probing. Mortality was determined at least three times using different collections of mosquitoes for each determination.

Low numbers of non-target species precluded determination of LC$_{50}$ concentrations. Instead, the insects were exposed to two concentrations of spinosad: 0.031 ppm, which is the LC$_{50}$ calculated in this study for field-collected mosquitoes, and 1.6 ppm, which is the published field rate for Natular®. Non-target species were used in bioassays on the same day they were collected from the site, and assays were conducted and data assessed similarly to the mosquito bioassays. The three taxa chosen as representative non-target organisms, based on their abundance at the collection site, were ultimate or penultimate instars of *Caenis* spp. (Ephemeroptera: Caenidae), *Ischnura* spp. (Odonata: Coenagrionidae), and *Pachydiplax longipennis* (Odonata: Libellulidae). For tests with *Caenis* spp., each determination consisted of 25 naiads divided into 5 dishes: four containing spinosad (at either 0.031 or 1.6 ppm) and one containing ddH$_2$O only (control). Susceptibility to each concentration was determined 5 times using naiads collected on different days. For assays with *Ischnura* spp. and *P. longipennis* one naiad was used per dish, due to known aggressive and cannibalistic behavior among naiads of *Ischnura* spp. and *P. longipennis*, respectively. Susceptibility to the two concentrations of spinosad was determined 3 times with each determination consisting of 5 controls and 5 treatments.

Statistics

Results from biological assays were corrected for control mortality (which never exceeded 10%) using Abbott's formula (Abbott, 1925), then subjected to probit analysis (Finney, 1952). Statistical differences between LC_{50} values were determined based on overlapping of 95% confidence intervals.

RESULTS

Survey of Mosquito Species

A total of 72 adult mosquitoes representing 12 species within 6 genera were collected from the study site (Table 1). The most abundant genus, in terms of individuals collected, was *Culex*, with 44 individuals. The most common species was *C. erraticus* (37 individuals), followed by *C. quinquefasciatus* (10 individuals). *Anopheles quadrimaculatus* was also present, with 9 individuals total, as well as four different species of *Aedes*, including *Ae. albopictus* and *Ae. vexans*, and a single *Ae. aegypti* and *Ae. triseriatus*. Several members of *Psorophora ferox* and *Uranotaenia sappharina* were also collected, as well as a single *Coquilettidia peturbans*.

Survey of Non-Target Taxa

Members of 14 families of insects from 5 orders were collected during a survey of the study site (Table 2). The three taxa with the highest overall abundance were naiads of *Ischnura* spp. (Odonata: Coenagrionidae; 38 individuals), *Pachydiplax longipennis* (Odonata: Libellulidae; 28 individuals) and *Caenis* spp. (Ephemeroptera: Caenidae; 29 individuals). These were then used as representative taxa in the non-target bioassays. In terms of diversity, Coleoptera were represented by the most families (5), including the genera *Peltodytes* and *Stenus* within the families Haliplidae and Staphylinidae, respectively, as well as members of families Scirtidae, Dytiscidae and Hydrophilidae. The order Diptera was represented predominantly by the family Chironomidae, and two individuals identified as either *Odontomyia* or *Hedriodiscus* (both in the family Stratiomyidae). Six individuals from different taxa in the order Hemiptera were also collected at different times of the year.

Table 1: Identities of adult mosquitoes collected. Numbers indicated mosquitoes collected per date traps were set and totals for each genus and species.

	6/8	6/12	6/16	6/22	Total Collected
Culex					**53**
quinquefasciatus	-	1	7	2	10
erraticus	13	-	1	23	37
salinarus	4	-	-	1	5
nigripalpus	1	-	-	-	1
Aedes					**16**
vexans	-	1	4	1	6
aegypti	-	-	1	-	1
albopictus	1	3	-	4	8
triseriatus	1	-	-	-	1
Anopheles quadrimaculatus	**9**	**-**	**-**	**-**	**9**
Psorophora ferox	**3**	**-**	**1**	**-**	**4**
Uranotaenia sappharina	**1**	**-**	**2**	**-**	**3**
Coquilettidia peturbans	**1**	**-**	**-**	**-**	**1**

Of the three representative taxa used for non-target bioassays, *Ischnura* spp. and *P. longipennis* were both present at the collection site year-round (Table 2) albeit in different abundances and life stages. However, greater numbers of *P. longipennis* were collected as the weather became cooler, with three samples on November 5th of 2011, Feb. 6th, and March 5th (average daily temperatures 55°F, 56°F, and 61°F, respectively) of 2012 yielding a total of 13 individuals (or 4.3 specimens per date) as compared to 6 individuals from 4 collections (or 1.5 specimens per date) during warmer weather on May 19th and 21st, June 27th and July 18th (average daily temperatures of 77°F, 79°F, 86°F, and 85°F) of 2012. Numbers of *Ischnura* spp., in comparison, varied widely throughout the year, with as many as 6 individuals collected on one day (May 24th, 2011), but none only 3 days later (May 27th). Members of *Caenis* spp. were abundant (i.e., 13 individuals) during the early to late spring months, but could not be found in November. These mayflies began to reappear in low numbers beginning in March of the following year and reached substantial numbers in May. Finally, whereas chironomids and the majority of

Table 2: Survey of non-target taxa.

	2011		2012			**Total**
	Spring	Fall	Winter	Spring	Summer	
<u>Coleoptera</u>						
Haliplidae-						
Peltodytes spp.	2	1				**3**
Scirtidae	7					**7**
Dytiscidae (adult)	1					**1**
Staphylinidae- *Stenus* sp.	1					**1**
Hydrophilidae (larval)	4					**4**
<u>Odonata</u>						
Coenagrionidae-						
Ischnura spp.	14	7	3	8	6	**38**
Enellagma spp.	1					**1**
Libellulidae-						
Pachydiplax longipennis	9	4	9	2	4	**28**
Miathyria sp. (?)	1					**1**
<u>Diptera</u>						
Chironomidae	14					**14**
Stratiomyidae-						
Odontomyia/Hedriodiscus	2					**2**
Ephemeroptera						
Caenidae- *Caenis* spp.	13		1		15	**29**
Hemiptera						
Mesoveliidae- *Mesovelia* spp.	2				1	**3**
Gerridae- *Limnoporus* spp.	2		1			**3**
Corixidae		2				**2**
Nepidae				1		**1**

* Numbers indicate the amount of individuals collected per season and year as well as totals per individual taxa. Collection dates were, in 2011: May 24th, 27th, 30th, June 1st, ,3rd, 7th, 9th, 22nd, November 5th, December 7th, and in 2012: February 6th, March 5th, May 19th, May 21st, June 27th, and July 18th.

coleopteran taxa were abundant in Spring of 2011, they were not found at the same time the following year.

Susceptibility of Culex quinquefasciatus *to Spinosad*

Based on overlap of confidence intervals, the susceptibility to spinosad did not differ between the laboratory reference strain (Sebring-S) and field collected mosquitoes (Fig. 5). Log dosage/probit relationships were linear and the LC_{50} values (and 95% confidence intervals) were 0.028 ppm (0.022-0.034) and 0.031 (0.02-0.047) ppm for the Sebring-S and field-collected mosquitoes, respectively.

Susceptibility to Spinosad in Non-target Organisms

Whereas susceptibility to spinosad in the non-target organisms varied depending on the species and concentration (Fig. 6), mortality was highest for *Caenis* spp. at 71% and 99% at concentrations corresponding to the LC_{50} for field-collected mosquitoes (0.031 ppm) and the field labeled rate (1.6 ppm), respectively. In tests with odonate species, there was no mortality among *Ischnura* spp. and only 6% mortality among *P. longipennis* at the mosquito LC_{50}. Mortality was higher at the field rate; 86% for *Ischnura* spp. but only 47% for *P. longipennis*.
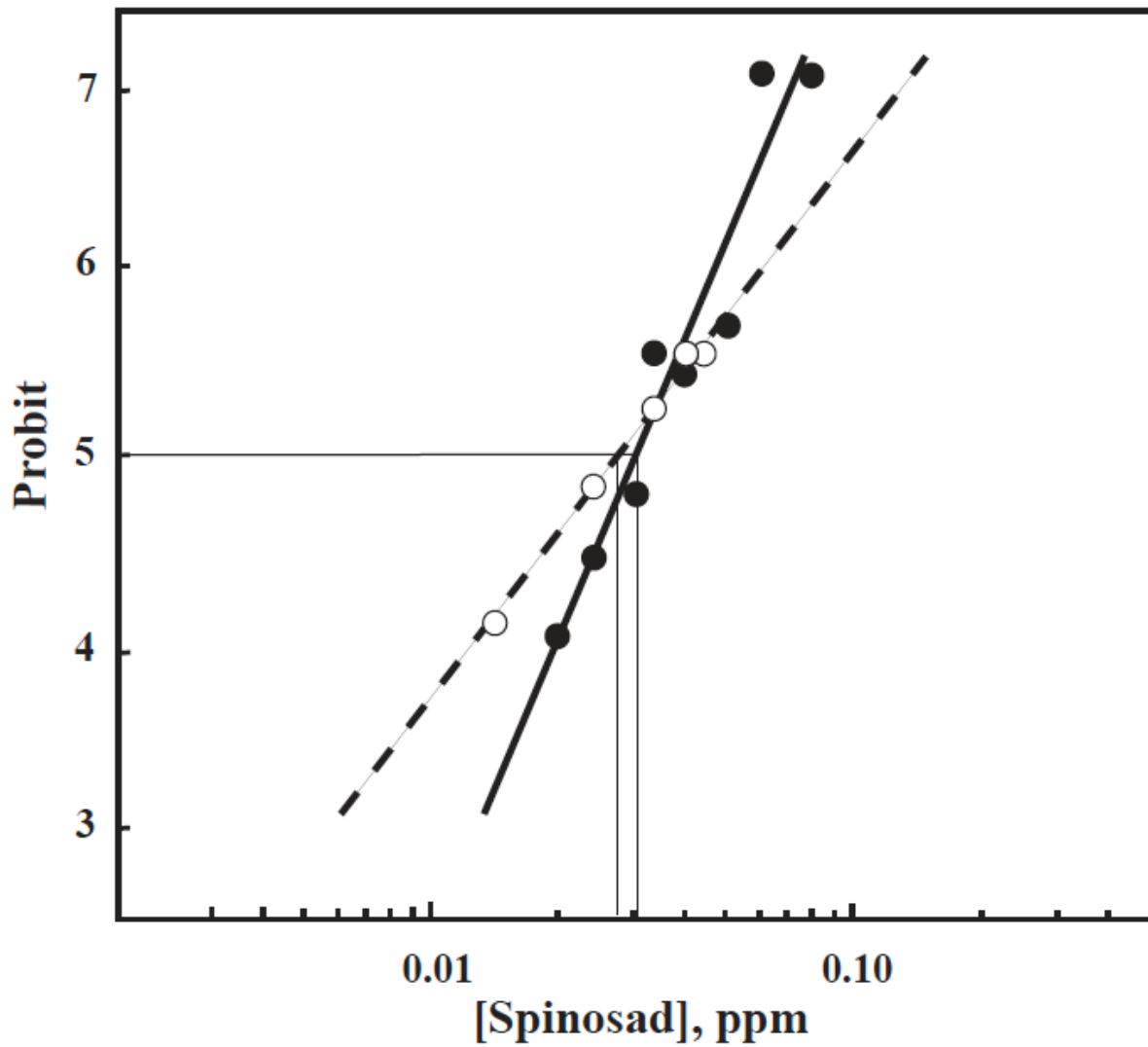
Fig. 5: Susceptibility to Spinosad in Sebring-S and Field-Collected *C. quinquefasciatus*. Sebring-S and Field-Collected populations are represented by open and closed circles, respectively.
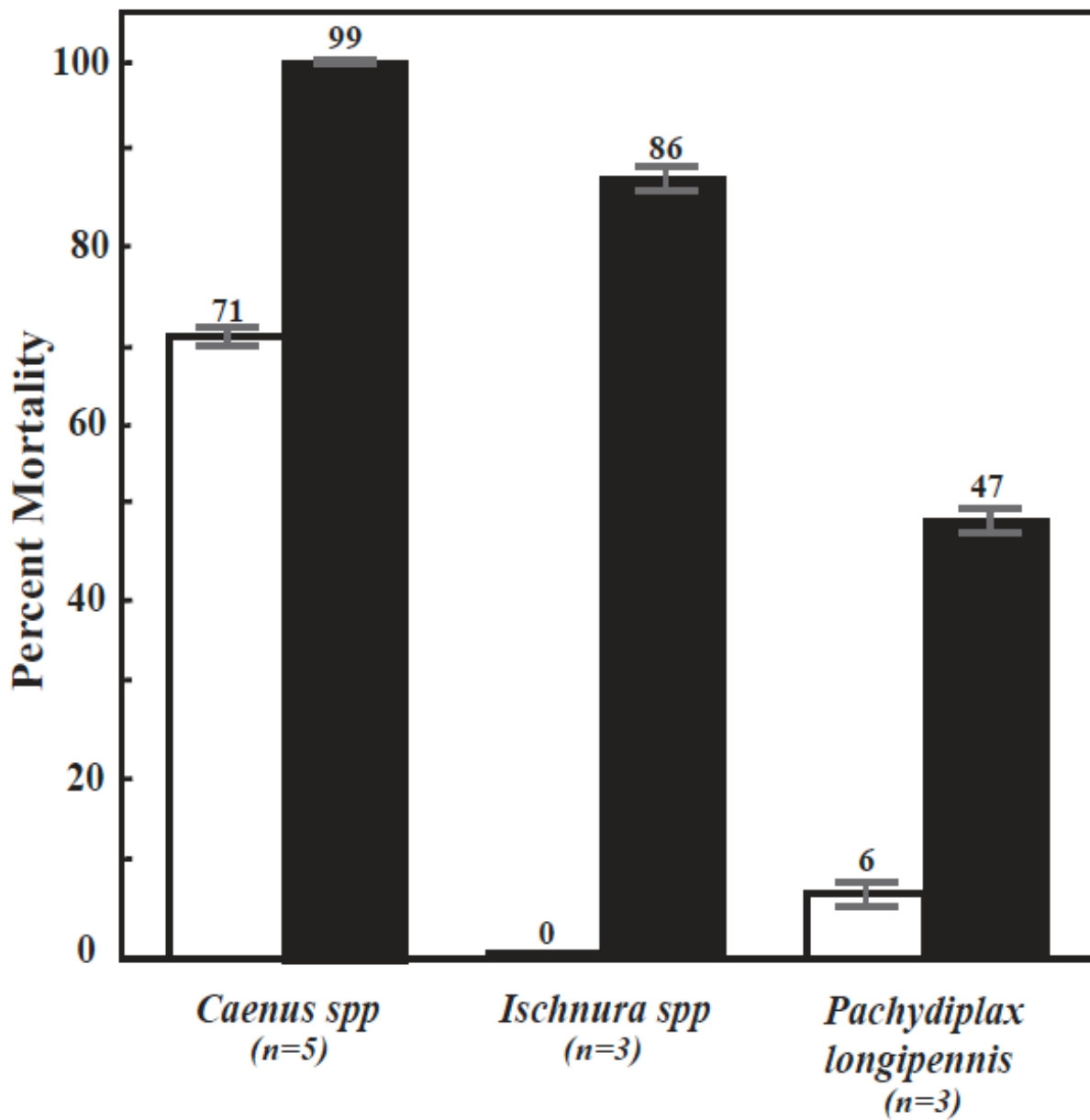
Fig. 6: Susceptibility of non-target organisms to spinosad. Percent mortalities for 0.031 ppm and 1.6 ppm are indicated by light and dark bars, respectively.

DISCUSSION

Members of the genus *Culex* were the most abundant mosquitoes found at the study site, and *C. erraticus* was the most abundant species. However, *C. quinquefasciatus* was studied further because it is present throughout the year in the southeastern U.S., as opposed to *C. erraticus*, which is more prominent during the summer. More importantly, *C. quinquefasciatus* is a major pest species capable of transmitting a number of debilitating diseases, including urban and lymphatic filariasis and West Nile. As such, it is currently the target of major control efforts in urban areas, some of which include the use of spinosad. Ten other mosquito species were found at the study site; however, many of these mosquitoes, such as *Ae. vexans*, *Ae. albopictus* and *C. salinarius*, have life histories that make it unlikely that the captured adults originated from the survey site. For example, *Ae. vexans* is a floodwater mosquito, while *Ae. albopictus* primarily breeds in containers, and *C. salinarius* prefer habitats with large amounts of rotting plant matter (Crans, 2004). These conditions were not present at the study site. Finally, a single specimen of *Ae. aegypti* was captured. It is worth noting that at the time of capture, this particular species was not known to have a locally established population within the Baton Rouge area (R. Vaeth, personal communication, 2011).

Susceptibility to spinosad, as measured in the current study, is similar to that reported previously in other studies of *C. quinquefasciatus* and other species of mosquitoes. Using similar methods but different instars (i.e., 2nd and 4th), formulations of spinosad (i.e., technical or suspension concentrate), and incubation period (48 hours), Hertlein et al. (2010) reported a range of LC_{50} values for *C. quinquefasciatus* (0.012-0.065 ppm) that is similar to values reported here. In addition, in previous studies, susceptibility to spinosad appears variable among different species of mosquitoes. In tests using technical spinosad, LC_{50} values were similar for *C.*

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quinquefasciatus and *Ae. aegypti* (0.065 ppm) but higher for *Ae. albopictus* (0.3 ppm) and lower

for *An. gambiae* (0.01 ppm). Finally, in the current study, no significant difference was observed

in susceptibility between lab-reared and field-collected mosquitoes. The LC_{50} ranges reported by

Hertlein et al. (2010) for field-collected *C. quinquefasciatus* (0.014 ppm - 0.023 ppm) are similar

to those measured in laboratory strains. However, direct comparisons of susceptibility to

spinosad between reference susceptible and wild populations of *C. quinquefasciatus* have not

been reported until now. Thus, the demonstration in the current study of the susceptibility of

field populations is valuable, and reinforces the utility of spinosad as a viable part of mosquito

control programs, especially in light of resistance to other insecticides in wild populations of *C.*

quinquefasciatus. For example, resistance to permethrin (up to 1,400-fold) in the U.S. and

elsewhere has been observed in *C. quinquefasciatus* (Chandre et al., 1998; Liu et al., 2009), as

has resistance to deltamethrin, malathion, chloropyrifos, fipronyl and imidacloprid (Liu et al.,

2004; 2009).

Effects of non-target exposure to spinosad have been examined in a number of species.

Spinosad is slightly to moderately toxic to fish with LC_{50}'s ranging from 5 ppm (for carp) to 30

ppm (for rainbow trout). In addition, spinosad is slightly toxic to aquatic invertebrates, with

LC_{50}'s ranging from 9.76 ppm (for grass shrimp, *Palaemonetes pugio*) to 92.7 ppm (for water

flea, *Daphnia magna*) (Dow Agrosciences, 2002). In the current study, susceptibility to spinosad

differed amongst the non-target taxa. *Caenis* spp. was the most susceptible although

susceptibility was comparable to that observed in field-collected mosquitoes. Odonates were

shown to be less susceptible, with negligible mortality measured at the lower dosage, (0 and 6%

for *Ischnura* spp., and *P. longipennis*, respectively). At the field-rate, however, mortality for

Ischnura spp. was very high (86%), but lower (47%) for *P. longipennis*. These differences in

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susceptibility may be due to weight, which was 10-fold lower for *Caenis* spp. relative to

Ischnura spp., and 10-fold lower for *Ischnura* spp. compared with *P. longipennis*.

A number of questions have arisen from this study. Many relate to examining non-target

effects of spinosad in a more comprehensive manner. For instance, susceptibility to spinosad

could differ depending on life stages. The three non-target taxa that were tested are aquatic as

immatures, but are all terrestrial as adults. In comparison, other insects (such as dytiscids) are

present in the same environment as both larvae and adults, and have very different life histories

(and possibly, differing susceptibilities). In addition, susceptibility of non-target taxa to spinosad

under different environmental conditions, such as water temperature, pH, salinity and organic

content, need to be studied, as these factors can alter the distribution and efficacy of spinosad.

Finally, an increased number of sampling techniques would allow for a more accurate

assessment of the occurrence of non-target fauna.

In summary, bioassays show that there is no significant difference in susceptibility to

spinosad between mosquitoes from local, field-collected populations and a lab-reared susceptible

reference strain of *C. quinquefasciatus*. This suggests a high degree of susceptibility in field

populations. In addition, non-targets species were also susceptible to spinosad. Mortality among

three representative species was variable and depended on dosage and species. A more

comprehensive study of the effects of spinosad on non-target organisms is needed.

This study may allow for development of improved pest management strategies with

spinosad. First of all, it provides an example of methods and materials that can be used to test

aquatic non-targets, which can serve as a basis for future studies. Second, the great difference

(several orders of magnitude) between the labeled field rate and the LC50's for the wild

populations and Sebring-S strain of *C. quinquefasciatus*, suggests that the application rate may be higher than what is actually necessary to manage populations of mosquito larvae. Reduced application rates of spinsoad may be adequate to control *C. quinquefasciatus* larvae while minimizing effects on non-target taxa.

LITERATURE CITED

Abbott, W.S. (1925). A Method for Computing the Effectiveness of an Insecticide. *J. Econ. Entomol.* **18**, 265-267.

Arnekleiv, J.V., Dolmen, D., Ronning. L. (2001). Effects of Rotenone Treatment on Mayfly Drift and Standing Stocks in Two Norwegian Rivers. *Trends in Research in Ephemeroptera and Plecoptera*. Ed. E. Dominguez. Kluwer Academic/Plenum Pub. **pp. 77-88**.

Bielza, P., Quinto V., Contreras, J., Torne M., Martin, A., Espinosa P.J.. (2007). Resistance to Spinosad in the Western Flower Thrips, *Frankliniella occidentalis* (Pergande), in Greenhouses of South-Eastern Spain. *Pest Manag. Sci.* **63**, 682-687.

Boellstorf, D.E., Ohlendorf, H.M., Anderson, D.W., O'Neill, E.J., Keith, J.O., Prouty, R.M. (1985). Organochloride Chemical Residues in White Pelicans and Western Grebes from the Klamath Basin, California. *Arch. Environ. Contam. Toxicol.* **14**, 485-493.

Bond, J.G., Marina, C.F., Williams, T. (2004). The Naturally Derived Insecticide Spinosad is Highly Toxic to *Aedes* and *Anopheles* Mosquito Larvae. *Med. Vet. Entomol.* **18**, 50-56.

Brown, A.W.A. (1986). Insecticide Resistance in Mosquitoes: a Pragmatic review. *J Am Mosq Control Assoc*.; **2**, 123-140.

Busby, A.J., Eckstein, G. (2009). Organophosphates, Friend and Foe: The Promise of Medical Monitoring for Farm Workers and Their Families. *J. Environ. Law*. **27**, 39-69.

Centers for Disease Control and Prevention. (2009). Saint Louis Encephalitis: Epidemiology & Geographic Distribution Retrieved June 2, 2012 from http://www.cdc.gov/sle/technical/epi.html

Centers for Disease Control and Prevention. (2010). The History of Malaria, an Ancient Disease. Retrieved August 23, 2011 from http://www.cdc.gov/malaria/about/history/

Centers for Disease Control and Prevention. (2011). West Nile Virus: Statistics, Surveillance, and Control Archive. Retrieved May 14, 2012 from http://www.cdc.gov/ncidod/dvbid/westnile/surv&control_archive.htm

Centers for Disease Control and Prevention. (2012). Final 2011 West Nile Virus Infections in the United States. Retrieved May 14, 2012 from http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount11_detailed.htm

Chandre, F., Darriet, F., Darder, M., Cuany, A., Doannio, J.M.C., Pasteur N., Guillet, P. (1998). Pyrethroid Resistance in *Culex quinquefasciatus* from West Africa. *Med. Vet. Entomol.* **12**, 359-366.

Chandre, F., Darriet, F., Doannio, J.M.C., Riviere, F., Pasteur, N., Guillet, P. (1997). Distribution of Organophosphate and Carbamate Resistance in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) in West Africa. *J. Med. Entomol*. **34**, 664-671.

Chareonviriyahpap, T., Aum-aung, B., Ratanatham, S. (1999). Current Insecticide Resistance Patterns in Mosquito Vectors in Thailand. *Southeast Asian J. Trop. Med. Public Health.* **30**, 184-194.

Cisneros, J., Goulson D., Derwent L.C, Penagos D.I., Hernandez, O., Williams T. (2002). Toxic Effects of Spinosad on Predatory Insects. *Biol. Control.* **23**, 156-163.

Cohen, Jr., M.M. (2001). Frog Decline, From Malformations, and a Comparison of Frog and Human Health. *Am. J. Med. Genet.* **104**, 101-109.

Cottam, C. (1965). The Ecologists' Role in Problems of Pesticide Pollution. *BioScience.* **15**, 457-463.

Crans, W.J. (2004). A Classification System for Mosquito Life Cycles: Life Cycle Types for Mosquitoes of the Northeastern United States. *J. Vector. Ecol.*

Creech, W.B. (1977). St. Louis Encephalitis in the United States, 1975. *J. Infect. Dis.* **135**, 1014-1016.

Darriet, F., Corbel, V. (2006). Laboratory Evaluation of Pyriproxyfen and Spinosad, Alone and in Combination, Against *Aedes aegypti* Larvae. *J. Med. Entomol.* **43**, 1190-1196.

Darriet, F., Duchon, S., Hougard, J.M. (2005). Spinosad: A New Larvicide Against Insecticide-Resistant Mosquito Larvae. *J. Am. Mosq. Cont. Assoc.*

Day, J.F. (2001). Predicting St. Louis Encephalitis Virus Epidemics: Lessons from Recent, and Not So Recent, Outbreaks. *Ann. Rev. Entomol.* **46**: 111-138.

Deepa, J. (2012). Role of Aquatic Insects as Biomonitors, Hussain Sagar Lake, Hyderabad. *Biological Forum- An International Journal.* Special Issue. **4**, 22-24.

Degitz, S.J. Durhan, E.J. Tietge, J.E., Kosian, P.A., Holcombe, G.W., Ankley, G.T. (2003). Developmental Toxicity of Methoprene and Several Degradation Products in *Xenopus laevis*. *Aquat. Toxicol.* **64,** 97-105.

Dewey, J.E. (1973). Accumulation of Fluorides by Insects Near an Emission Source in Western Montana. *Environ. Entomol.* **2**, 179-182.

Dow AgroSciences, (1998). Material Safety and Data Sheet: Tracer(R) Naturalyte Insect control. Dow AgroSciences, Indianapolis, IN.

Dow AgroSciences (2002). Material Safety and Data Sheet: Entrust* Naturalye* Insect Control. Dow AgroSciences, Indianapolis, IN.

Duchet, C., Caquet, Th., Franquet, E., Lagneau, C., Lagadic, L. (2010a). Influence of Enviormental Factors on the Response of a Natural Population of *Daphnia magna* (Crustacea: Cladocera) to Spinosad and *Baciluus thuringiensis israelensis* in Mediterranean Coastal Wetlands. *Env. Poll.* **158**, 1825-1833.

Duchet, C., Coutellec, M-A., Franquet, E., Lagneau, C., Lagadic, L. (2010b). Population-Level Effects of Spinosad and *Bacillus thuringiensis israelensis* in *Daphnia pulex* and *Daphnia magna*: Comparison of Laboratory and Field Microcosm Exposure Conditions. *Ecotoxicology*. **19**, 1224-1237.

Duchet, C., Larroque, M., Caquet, Th., Franquet, E., Lagneau, C., Lagadic, L. (2008). Effects of Spinosad and *Bacillus thuringiensis israelensis* on a Natural Population of *Daphnia pulex* in Field Microcosms. *Chemosphere*. **74**, 70-77.

Eger Jr., J.E., Stavisky, J., Funderburk, J.E. (1998) Comparative Toxicity of Spinosad to *Frankliniella* spp. (Thysanoptera: Thripidae), With Notes on a Bioassay Technique. *Fla Entomol*. **81**, 547-551.

Fialkowski, W., Klonowska-Olejnik, M., Smith, B.D., Rainbow, P.S. (2003). Mayfly Larvae (*Baetis rhodani* and *B. vernus*) as Biomonitors of Trace Metal Pollution in Streams of a Catchment Draining a Zinc and Lead Mining Area of Upper Silesia, Poland. *Environ. Pollut.* **121**, 253-267.

Finney, D.J. (1952). *Probit Analysis*. 2nd ed. Cambridge: University Press.

Flieschli, M.A., Franson, J.C., Thomas, N.J., Finley, D.L., Riley, Jr. W. (2004). Avian Mortality Events in the United States Caused by Anticholinesterase Pesticides: A Retrospective Summary of National Wildlife Health Center Records from 1980 to 2000. *Arch. Environ. Contam. Toxicol.* **46**, 542-550.

Georghiou, G.P., Ariaratnam, V., Pasternak, M.E., Lin, C.S. (1975). Organophosphorous Multiresistance in *Culex pipiens quinquefasciatus* in California. *J. Econ Entomol*. **68**, 461-467.

Gericke, A., Govere, J.M., Durrheim, D.N. (2002). Insecticide Susceptibility in the South African Malaria Mosquito *Anopheles arabiensis* (Diptera: Culicidae). *S. Afr. J. Sci.* **98**, 205-208.

Gray, R.H. (1974). The Decline of Mortality in Ceylon and the Demographic Effects of Malaria Control. *Popul. Stud*. **28**: 205-229.

Goldberg, T.L., Anderson, T.K., Hamer. G.L. (2010) West Nile Virus May Have Hitched a Ride Across the Western United States on *Culex tarsalis* Mosquitoes. *Mol. Ecol*. **19**, 1518-1519.

Hatakeyama, S., Shiraishi, H., Uno S. (1997). Overall Pesticide Effects on Growth and Emergence of Two Species of Ephemeroptera in a Model Stream Carrying Pesticide-Polluted River Water. *Ecotoxicology*. **6**, 167-180.

Hemingway, J., Ranson, H. (2000). Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology*. **45**, 371-391.

Hertlein, M.B., Mavrotas, C., Jousseaume, C., Lysandrou, M., Thompson, G.D., Jany, W., Ritchie, S.A. (2010). A Review of Spinosad as a Natural Product for Larval Mosquito Control. *J. Mosq. Cont. Assoc.* **26**, 67-87

Housset, P., Dickmann, R. (2009). A Promise Fulfilled- Pyrethroid Development and the Benefits for Agriculture and Human Health. *Bayer Crop Science Journal.* **62**, 135-144.

Huang, G., Vergne, E., Gubler, D.J. (1992). Failure of Dengue Viruses to Replicate in *Culex quinquefasciatus* (Diptera Culicidae). *J. Med. Entomol.* **29**, 911-914.

Hunt, K.A., Bird, D.M., Mineau, P., Shutt, L. (1991). Secondary Poisoning Hazard of Fenthion to American Kestrels. *Arch. Environ. Contam. Toxicol.* **21**, 84-90.

Johnsen, M.M. "The Status of Resistance in *Culex quinquefasciatus* Say (Diptera: Culicidae) Populations in Brazos and Harris Counties, Texas." Diss. Texas A&M University, Dept. of Entomology. 2007.

Johnson, B.T., Saunders, C.R., Sanders, H.O., Campbell, R.S. (1971). Biological magnification and degradation of DDT and Aldrin by freshwater invertebrates. *J. Fish. Res. Bd. Can.* **28**, 705-709.

Khan, M.Z., Law, F.C.P. (2005). Adverse Effects of Pesticides and Relate Chemicals on Enzyme and Hormone Systems of Fish, Amphibians and Reptiles: A Review. *Proc. Pakistan Acad. Sci.* **42**, 315-323.

Killeen, G.F., Seyoum,, A., Knols, B.G.J. (2004). Rationalizing Historical Successes of Malaria Control in Africa in Terms of Mosquito Resource Availability Management. *Am. J. Trop. Med. Hyg.* **71 (Supplemental Issue 2)**, 87-93.

Landa, V., Soldan, T. (1995). Mayflies as Bioindicators of Water Quality and Environmental Change on a Regional and Global Scale. *Current Directions in Research on Ephemeroptera.* **pp. 21-29**.

Legocki, J., Polec, I., Zelechowski, K. (2010). Contemporary Trends in Development of Active Substances Possessing the Pesticidal Properties: Spinosyn Insecticides. *Pesticides.* **1**, 59-71.

Liu, H., Cupp, E.W., Micher, K.M., Guo, A., Liu, N. (2004). Insecticide Resistance in Alabama and Florida Mosquito Strains of *Culex quinquefasciatus*. *J. Med. Entomol.* **41**, 408-413.

Liu, N., Xu, Q., Li, T., He, L., Zhang, L. (2009). Permethrin Resistance and Target Site Insensitivity in the Mosquito *Culex quinquefasciatus* in Alabama. *J. Med. Entomol.* **46**, 1424-1429.

Magnin, M., Marboutin, E., Pasteur, N. (1988). Insecticide Resistance in *Culex quinquefasciatus* (Diptera: Culicidae) in West Africa. *J. Med. Entomol.* **25**, 99-104.

Marzaro, M., Viva, L., Targa, A., Mazzon, L., Mori, N., Greatti, M., Toffolo, E.P., Di Bernardo, A., Giorio, C., Marton, D., Tapparo, A., Girolami, V. (2011). Lethal Aerial Powdering of Honey Bees With Neonicotinoids from Fragments of Maize Seed Coat. *Bull. Insectol.* **64**, 119-126.

Mathis, B.J., Cummings, T.F., Gower, M., Taylor, M., King, C. (1977). Dynamics of Manganese, Cadmium, and Lead in Experimental Power Plants Ponds. Water Research Center, Univ. Illinois. [OdA 2267].

Maund, S.J., Hamer, M.J., Lane, C.G., Farrelly, E., Rapley, J.H., Goggin, U.M., Gentle, W.E. (2002). Partitioning, Bioavailability, and Toxicity of the Pyrethroid Insecticide Cypermethrin in Sediments. *Environ. Toxicol. Chem.* **21**, 9-15.

Medina, P., Morales, J., Smagghe, G., Vinuela, E. (2008). Toxicity and Kinetics of Spinosad in Different Developmental Stages of the Endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) and its Host *Spodoptera littoralis* Larvae (Lepidoptera: Noctuidae). *Biocontrol.* **53**, 569-578.

Menetrey, N., Oertli, B., Sartori, M. Wagner, A., Lachavanne, J.B. (2008). Eutrophication: Are Mayflies (Ephemeroptera) Good Bioindicators for Ponds? *Hydrobiologia.* **597**, 125-135.

Meyer, W., Harisch, G., Sagredost, A.N. (1986). Biochemical Aspects of Lead Exposure in Dragonfly Larvae (Odonata: Anisoptera). *Ecotoxicol. Environ. Safety.* **11**: 308-319.

Mielewczyk, S. (1977). VIII. Odonata. In Wroblewski, A. (ed.), "Bottom Fauna of the Heated Konin Lakes." pp. 205-212. Monografie Fauny Polski 7, Warsaw.

Miskus, R.P., Blair, D.P., Casida, J.E. (1964). Conversion of DDT to DDD by Bovine Rumen Fluid, Lake Water, and Reduced Porphyrins. *J. Agr. Food Chem.* **13**, 481-483.

Mortimer, M.R., Chapman, H.F. (1995). Acute Toxic Effects of (S)-Methoprene and Temephos to Some Australian Non-Target Aquatic Crustacean Species. *Australian Journal of Exotoxicology.* **1**, 107-111.

Murihead-Thompson, R.C. (1971). Pesticides and Freshwater Fauna. Academic Press, New York.

Murray, K., Walker, C., Herrington, E., Lewis, J.A., McCormick, J., Beasley, D.W.C., Tesh, R.B., Fisher-Hoch, S. (2010). Persistent Infection with West Nile Virus Years after Initial Infection. *J. Infect. Dis.* **201**, 2-4.

Myung , K., Massoughbodji, A., Ekoue, S., Atchade, P., Kiki-Fagla, V., Klion, A.D. (1998). Lymphatic Filariasis in a Hyperendemic Region: A Ten-Year, Follow-up Panel Survey." *Am. J. Trop. Med. Hyg.* **59**, 222-226.

Needham, J.G., Westfall, M.J., May, M.L. (2000). Dragonflies of North America. Gainesville, Fl. Scientific Publishers, Inc.

Nevskaia, D.M., Castillejos-Lopez, E., Guerrero-Ruiz, A., Munoz, V. (2004). Effects of the Surface Chemistry of Carbon Materials on the Adsorption of Phenol-aniline Mixtures from Water. *Carbon.* **42**, 653-665.

Perez, C.M., Marina, C.F., Bond, J.G., Rojas, J.C., Valle, J., Williams, T. (2007). Spinosad, a Naturally Derived Insecticide, for Control of *Aedes aegypti* (Diptera: Culicidae): Efficacy Persistence and Elicited Oviposition Response." *J. Med. Entomol.* **44**, 631-638.

Peterson, J.L., Jepson, P.C., Jenkins, J.J. (2001). Effect of Varying Pesticide Exposure Duration and Concentration on the Toxicity of Carbaryl to Two Field-Collected Stream Invertebrates,

Calineuria californica (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae). *Environ. Toxicol. Chem.* **20**, 2215-2223.

Priester, T., Georghiou, G.P. (1978) Induction of High Resistance to Permethrin in *Culex pipiens quinquefasciatus*. *J. Econ. Entomol.* **71**, 197-200.

Ravichandran, S. (2011). Green Chemistry for Sustainable Development. *Asian J. Biochem. Pharm. Res*. 2011. **1**, 129-135.

Regis, L., Oliveira, C.M.F., Silva-Filha, M.H., Silva, S.B., Maciel, A., Furtado, A.F. (2000). Efficacy of *Bacillus sphaericus* in Control of the Filariasis Vector *Culex quinquefasciatus* in an Urban Area of Olinda, Brazil. *T. Roy. Soc. Trop. Med. H*. **94**, 488-492.

Rosen, L., Roseboom, L.E., Gubler, D.J., Lien, J.C., Chaniotis, B.N. (1985). Comparative Susceptibility of Mosquito Species and Strains to Oral and Parenteral Infection with Dengue and Japanese Encephalitis Viruses. *Am. J. Trop. Med. Hyg*. **34**, 603-615.

Roth, D., Henry, B., Mak, S., Fraser, M., Taylor, M., Li, M., Cooper, K., Furnell, A., Wong, Q., Morshed, M. (2010). Members of the British Columbia West Nile Virus Surveillance Team. West Nile Virus Range Expansion into British Columbia. *Emerg. Infect. Dis* **16**, 1251-1258.

Rupperecht, R. (1975). The Dependence of Emergence-Period in Insect Larvae on Water Temperature. *Verth. Int. Verein. Limnol.* **19,** 3057-3063.

Salgado, V.L. (1998). Studies on the Mode of Action of Spinosad: Insect Symptoms and Physiological Correlates. *Pestic. Biochem. Phys*. **60**, 91-102.

Salgado, V.L., Sheets, J.J., Watson, G.B., Schmidt, A.L. (1998). Studies on the Mode of Action of Spinosad: The Internal Effective Concentration and the Concentration Dependence of Neural Excitation. *Pestic. Biochem. Phys.* **60**, 103-110.

Sbrana, E., Tonry, J.H., Xiao, S-Y, da Rosa, A.P.A.T., Higgs, S., Tesh, R.B. (2005). Oral Transmission of West Nile Virus in a Hamster Model. *Am. J. Trop. Med. Hyg.* **72**, 325-329,

Shoaib, N., Siddiqui, P.J.A., Ali, A. (2012). Acute Toxic Effects of Organophosphate Pesticides on Killifish Fish (*Aphanius dispar*) Juveniles. *Pakistan J. Zool.* **44**, 569-572.

Shono, T., Scott, J.G. (2003). Spinosad Resistance in the Housefly, *Musca domestica*, is Due to a Recessive Factor on Autosome 1. *Pestic. Biochem. Phys*. **75**, 1-7.

Siegfried, B.D. (1993). Comparative Toxicity of Pyrethroid Insecticides to Terrestrial and Aquatic Insects. *Environ. Toxic. Chem.* **12**, 1683-1689.

Snow, R.W., Craig, M., Deichmann, U., Marsh, K. (1999). Estimating Mortality, Morbidity and Disability Due to Malaria Among Africa's non-Pregnant Population. *B. World Health Organ.* **77**, 624-640.

Snyder, D.E., Meyer, J., Zimmermann, A.G., Qiao, M., Gissendanner, S.J., Cruthers, L.R., Slone, R.L., Young, D.R. (2007). Preliminary Studies on the Effectiveness of the Novel

Pulicide, Spinosad, for the Treatment and Control of Fleas and Dogs. *Vet. Parasitol.* **150**, 345-351.

Soldan, T. (1981). Comparative Anatomy of the Internal Reproductive Organs of Mayflies (Ephemeroptera). *Studie Sav 6.* Praha: Academia

Stapleton, D.H. (2004). Lessons of History? Anti-Malaria Strategies of the International Health Board and the Rockefeller Foundation from the 1920s to the Era of DDT. *Public Health Rep.* **119,** 206-215.

Stark, J.D., Vargas, R.I. (2003). Demographic Changes in *Daphnia pulex* (leydig) After Exposure to the Insecticides Spinosad and Diazinon. *Ecotoxicol. Environ. Safety.* **56**, 334-338.

Stone, W.B. Overmann, S.R., Okoniewski, J.C. (1984). Intentional Poisoning of Birds with Parathion. *The Condor.* **86**, 333-336.

Subramanian, M.A., and Varadaraj, G. (1993). The Effect of Industrial Effluents on Moulting in *Macromia cingulata* (Rambur) (Anisoptera: Coreduliidae). *Odonatologica.* **22**, 229-232.

Sudia, W.D., and Chamberlain, R.W. (1962). Battery-Operated Light Trap, An Improved Model. *Mosquito News.* **22**, 126-129.

Tang, J.-X., Siegfried, B.D. (1995). Comparative Uptake of a Pyrethroid and Organophosphate Insecticide by Selected Aquatic Insects. *Bull. Environ. Contam. Toxicol.* **55**, 130-135.

Thanispong, K., Sathantrithop, S., Chareonviriyaphap, T. (2008). Insecticide Resistance of *Aedes aegypti* and *Culex quinquefasciatus* in Thailand. *J. Pest. Sci.* **33**, 351-356.

Thompson, G.D., Dutton, R., Sparks, T.C. (2000). Spinosad- a Case Study: An Example From a Natural Products Discovery Programme. *Pest Manage. Sci.* **56**, 696-702.

Triteeraprabab, S., Kanjanopas, K., Suwannadabba, S., Sangprakarn, S., Poovorawan, Y. (2000). Transmission of the Nocturnal Periodic Strain of *Wucheria bancrofti* by *Culex quinquefasciatus*: Establishing the Potential for Urban Filariasis in Thailand. *Epidemiol. Infect.* **125**, 207-212.

United States Environmental Protection Agency. Office of Pesticides and Toxic Substances. Pesticide Fact Sheet: Spinosad.. 1999.

Utzinger, J., Tozan, Y., Doumani, F., Singer, B.H. (2002). The Economic Payoffs of Integrated Malaria Control in the Zambian Copperbelt Between 1930 and 1950. *Trop. Med. Int. Health.* **7**, 657-677.

Vazeille-Falcoz, M., Rosen, L., Mousson, L., Rodhain. F., (1999). Replication of Dengue Type 2 Virus in *Culex quinquefasciatus* (Diptera: Culicidae). *Am. J. Trop. Med. Hyg.* **60**, 319-321.

Verslycke, T., Ghekiere, A., Raimondo, S., Jansse, C. (2007). Mysid Crustaceans as Standard Models for the Screening and Testing of Endocrine Disrupting Chemicals. *Exotoxiocology.* **16**, 205-219.

Weaver., S.C., Reisen, W.K. (2010). Present and Future Arboviral Threats. *Antivir. Res.* **85**, 328-345.

West, S.D., Yeh, L-T., Turner, L.G., Schwedler, D.A., Thomas, A.D., Duebelbeis, D.O. (2000). Determination of Spinosad and its Metabolites in Food and Environmental Matrices. 1. High-Performance Liquid Chromatography with Ultraviolet Detection. *J. Agric. Food Chem.* **48**, 5131-5137.

Westfall, Jr., M.J., May, M.L. (1996). Damselflies of North America. Gainesville, FL: Scientific Publishers Inc.

White, D.H., King, K.A., Mithcell, C.A., Hill, E.F., Lamont, T.G. (1979). Parathion Causes Secondary Poisoning in a Laughing Gull Breeding Colony. *Bull. Environm. Contam. Toxicol.* **23**, 281-284.

Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D. (2012). Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. *Science*. **336**, 351-352.

Williams, T., Valle, J., Vinuela, E. (2003). Is the Naturally Derived Insecticide Spinosad (R) Compatible with Insect Natural Enemies? *Biocontrol Sci. Techn*. **5**, 459-475.

Wixson, B.G., and Clark, W.J. (1967). Gamma Radiation Effects on Nymphs of *Argia translata*. *Ann. Entomol. Soc. Am.* **60**, 485-486. [BsA 62492].

WHO (1976). Resistance of Vectors and Reservoirs of Disease to Pesticides. 22nd Rep. WHO Expert Committee on Insecticides. *Tech. Rep. Ser*. **585**, 88

WHO (1980). Resistance of Vectors and Reservoirs of Disease to Pesticides. Fifth Rep. WHO Expert Committee on Vector Biology and Control. *Tech. Rep. Ser.* **655**, 82

WHO (1986). Resistance of Vectors and Reservoirs of Disease to Pesticides. Tenth Report of the WHO Expert Committee on Vector Biology and Control. *Tech. Rep. Ser.* **737**, 87

WHO (1992). Vector Resistance to Pesticides. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. *Tech. Rep. Ser.* **818**, 1

WHO (2009). Weekly Epidemiological Record (13 March, 2009). **84**, 85-96.

WHO (2010). WHO Global Malaria Programme: World Malaria Report 2010. *WHO Press*.

WHO (2011). WHO Global Malaria Programme: World Malaria Report 2011. *WHO Press.*

Wolanksy, M.J., Harrill, J.A. (2008). Neurobehavioral Toxicology of Pyrethroid Insecticides in Adult Animals: A Critical Review. *Neurotoxicology and Teratology*. **30**, 55-78.

Yang, E.C., Chuang, Y.C., Chen, Y.L., Chang, L.H. (2008). Abnormal Foraging Behavior Induced by Sublethal Dosage of Imidacloprid in the Honey Bee (Hymenoptera: Apidae). *J. Econ. Entomol.* **101**, 1743-1748.

Zhao, J.-Z., Li, Y-X., Collins, H.L., Gusukuma-Minuto, L., Mau, R.F.L., Thompson, G.D., Shelton, A.M. (2002). Monitoring and Characterization of Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Spinosad. *J. Econ. Entomol.* **95**, 430-436.

VITA

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