Journal of the Lepidopterists' Society
55(1), 2001, 8–14

INFLUENCE OF MOSQUITO CONTROL CHEMICALS ON BUTTERFLIES (NYMPHALIDAE, LYCAENIDAE, HESPERIIDAE) OF THE LOWER FLORIDA KEYS

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ABSTRACT. A 14-month survey was conducted in the rock pinelands of south Florida (Long Pine Key) and the Lower Florida Keys (Big Pine Key) to determine the status of three potentially threatened butterfly species. Populations of the Florida leafwing, *Anaea troglodyta floridalis* F. Johnson & Comstock, and Bartram's hairstreak, *Strymon acis bartrami* (Comstock & Huntington), were monitored in areas that receive year-round chemical applications for mosquito control and in those without such treatment. *Anaea troglodyta floridalis* maintained significantly higher adult densities during both years of the survey on transects where chemical applications were restricted. No significant differences were found in *A. troglodyta floridalis* larval densities among transects in either year; however, the overall larval density was significantly higher in the 1998 sampling period. *Strymon acis bartrami* showed consistently high adult and larval densities at all Big Pine Key transects, but was not observed in Long Pine Key. A third species, the rock-land grass skipper *Hesperia meskei* (W. H. Edwards), was not found on any of the survey transects despite a high density of its host grass, *Aristida purpurascens* Poir. (Poaceae). Experiments to test the potential toxicity of mosquito control chemicals on various surrogates of the above species showed naled and permethrin to be most toxic, with lethal dosage (LD_{50}) values of 1.0 µg or less of AI per gram of body weight for the species and stages tested. LD_{50} values of 48.1 µg or less AI per gram of body weight were found for surrogates treated with malathion. Given the susceptibility of these butterflies in all their life stages to the mosquito control insecticides presently in use, these chemicals should be considered a major factor in the populational declines and fluctuations of the butterflies studied.

Additional key words: *Anaea,* chemical pesticides, non-target arthropods, *Strymon, Hesperia.*

Human activity in south Florida and the Florida Keys has increased dramatically in recent decades. With year-round mild climate and scenic beauty, the area became an appealing place to live and visit. It was only a matter of time until this region's unique flora and fauna would feel the effect of human population growth. Among the first to be negatively influenced were the native butterflies. Although normally more appreciated for their aesthetic appeal, butterflies are also an extremely good indicator of an ecosystem's stability (Erhardt 1985, Longley & Sotherton 1997). *Anaea troglodyta floridalis,* the Florida leafwing F. (Johnson & Comstock) (Nymphalidae), *Strymon acis bartrami,* the Bartram's hairstreak (Comstock & Huntington) (Lycaenidae) and the rock-land grass skipper, *Hesperia meskei* (W. H. Edwards) (Hesperiidae) have enjoyed relatively large historic ranges, occupying the pinelands that once covered most of southern Florida and the Lower Keys (Minno & Emmel 1993, Smith et al. 1994). Their rapid demise in recent decades (Baggett 1982, Schwartz 1987, Hennessey & Habeck 1991, Schwarz et al. 1995, Salvato 1999) is representative of many species in the region and can be attributed in large part to habitat loss and mismanagement.

Another possible contributor to the decline of these butterflies is the use of chemical pesticides meant to control mosquitoes but with collateral effects on non-target arthropod species. The lethal effect of second-generation organophosphate pesticides, such as naled and fenthion, on non-target Lepidoptera was particularly well noted initially in south Florida and the Keys, with the demise of the Schaus' Swallowtail, *Papilio aristodemus ponceanus* Schaus (Emmel & Tucker 1991, Eliazar 1992). This species' dramatic decline in the early 1970s coincided with the expanded use of chemical pesticides by the Monroe County Mosquito Control District (MCMCD) on the northern Keys. When spraying was halted during two periods (1987 and 1989–1992), the species began to recover. Its immediate decline when applications resumed clearly suggested the adverse effect that chemical pesticides were having on non-target species. Baggett (1982) suggested that the rapid decline in *A. troglodyta floridalis* and *S. acis bartrami* populations on the Lower Keys was directly attributable to mosquito control insecticide applications.

Studies conducted by Hennessey et al. (1991, 1992) illustrated the presence of spray residue long after application in the habitat of the Schaus' Swallowtail and several other threatened butterflies, including *A. troglodyta floridalis* and *S. acis bartrami.* This research followed a joint agreement between U.S. Fish & Wildlife and MCMCD in 1987 on areas which were to be designated "no-spray" zones. Dade County did not spray insecticides for mosquito control during that time period. Thus the only chance for chemical contact on the southern mainland for these butterfly species occurs in the residential areas east of Long Pine Key, in Everglades National Park, where resmethrin is sprayed occasionally. As of 1989 the following areas in the Florida Keys were designated no-spray zones by agreement between U.S. Fish & Wildlife and MCMCD: in the north, a strip of land

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east of Crocodile Lake National Wildlife Refuge, Elliott Key, and several of the smaller keys of Biscayne National Park; and in the Lower Keys, the small outlying areas of the National Key Deer Wildlife Refuge. All of Big Pine Key except Watson's and eastern Cactus Hammocks is sprayed with the chemical pesticides naled, permethrin and *Bacillus thuringiensis* var. *israeliensis*.

However, no-spray does not mean a lack of chemical intrusion. These areas were established with the understanding that there was to be no use of insecticides, and any residues detected within them would be unacceptable. When these zones were created, pesticide drift downwind into them had not been documented. Hennessey et al. (1992) documented naled residues from the edge of Watson's Hammock on α-cellulose pads up to six hours after its application. The highest EEC recorded was 0.009 ± 0.0001 $\mu g/cm^2$ as compared to 0.011 ± 0.001 $\mu g/cm^2$ in the target area. Residues were detected 750 m into the no spray zone at Watson's Hammock, 150 m at Cactus Hammock and 30 m into the Schaus' hardwood hammock habitat on Key Largo's Crocodile Lake. Truck-applied ultra-low-volume (ULV) fenthion, sprayed primarily in residential areas, did not appear to drift into non-target areas. This study indicated that naled remained in the habitat well into midday, posing risk to diurnally active non-targets such as the Florida leafwing, rock-land grass skipper and Bartram's hairstreak.

Eliazar (1992) conducted intensive testing on the effects of the chemical pesticides naled and fenthion on several south Florida non-target nymphalid and papilionid species. His results indicated that chemical pesticides and their field application rates, particularly those of naled, were indeed extremely toxic to non-target Lepidoptera, and were being administered in the field at levels above the dosage required to kill target *Aedes* mosquitoes. Eliazar's naled experiments included several butterfly species likely to be found in the Lower Keys, including nymphalid species similar to the Florida leafwing. Among these were the gulf fritillary, *Agraulis vanillae* Michener, and the zebra longwing, *Heliconius charitonius* Comstock & Brown.

The potential influence of the pyrethroids, such as permethrin, which are currently used in the Lower Keys has only been evaluated for two butterfly species previously, *Papilio cresphontes* Cramer (Papilionidae) and *Vanessa caudui* L. (Nymphalidae) (Eliazar 1991, 1992). Furthermore, toxicity tests have never been performed to determine the effect of any chemical pesticide upon lycaenids, such as Bartram's hairstreak, or on hesperiids such as the rock-land grass skipper. Part of any recovery plan for these species must include evaluation of pesticide effects on populations. I conducted experiments on various life stages of non-threatened butterflies (congeneric to the three focal species here), to obtain lethal toxicity levels for exposure to naled, malathion and permethrin. Furthermore, I monitored populations of Florida leafwing, rock-land grass skipper and Bartram's hairstreak in the field, and examined the possible impact of chemicals being sprayed for control of adult mosquitoes in the Lower Keys.

MATERIALS AND METHODS

Population survey. A 14-month survey was conducted in south Florida and the Lower Florida Keys from July 1997 to August 1998. Line transects (N = 9) were established on Big Pine Key to monitor populations of *Anaea troglodyta floridalis, Strymon acis bartrami* and *Hesperia meskei*. The survey employed a combination of several butterfly count methods (Pollard 1977, Gall 1995). These were adapted to accommodate the sparse populations of butterflies associated with a consistently occurring host on Big Pine Key. Each transect was 400 m in length × 5 m in width (area 0.2 ha) (437 × 6 yards, or 0.5 acres); each had evenly distributed amounts of *Croton linearis* Jacq., the sole host plant for both *A. troglodyta floridalis* and *S. acis bartrami. Croton linearis* (N = 100) of varying sizes were randomly chosen within each transect and marked with flagging tape for larval inspection. These plants were surveyed at every visit during the course of the study. When plants died, new ones were included. Larvae and adults viewed at the fringes of designated transects were noted but not included in transect counts. *Aristida purpurascens*, the only known host plant for *H. meskei*, was inspected for larval activity. Transects were visited twice daily during late spring to early fall (April–August 1998, July–September 1997), and once daily the rest of the year (October–December 1997, January–March 1998) when daylight was more limited. This procedure allowed mainland and key transects to be visited on the same sampling dates. One transect was established at Gate 4 of Long Pine Key within Everglades National Park; it had the same dimensions as described above, but it was interrupted by a clear-cut area from historical logging times which mimicked grass savannah at its midpoint, and this clear-cut area was not considered part of the transect proper. Gate 4 at LPK was chosen because its pineland habitat is extremely similar to that of Big Pine Key. These two areas historically have maintained the largest populations of *A. troglodyta floridalis* and *S. acis bartrami* as well as moderate levels of *H. meskei*. The pinelands of Everglades National Park are not sprayed with chemical pesticides.

Anaea troglodyta floridalis and *S. acis bartrami* adults were captured by net and marked with the 1-2-4-7 numbering system (Ehrlich & Davidson 1960). *Hesperia meskei* was not observed within the transects at any point during the survey despite high density of its host. Plants which contained earlier stages were marked with field tape for later inspection; however, this technique was replaced by use of natural field markers whenever possible.

Adult and larval densities were transformed to the square root of (X + 0.5) and analyzed by ANOVA: Single Factor analysis in a completely randomized block design with all sampling dates and sites as sources of variance. Data from 1997 and 1998 were analyzed separately. All treatment areas were compared against controls (Watson's Hammock and Long Pine Key, the areas where insecticide applications are restricted). Where significant F values were found, Tukey's test was used to separate means.

Lethal dosage experiments. To help determine if mosquito control chemicals have a toxic effect on the various life stages of these potentially threatened butterfly species, lethal dosage levels were determined for similar, non-threatened butterfly species. The chemical insecticides used were malathion (O, O-dimethyl phosphorodithioate of diethyl mercaposuccinate; molecular weight = 330.4), naled (dimethyl-1, 2-dibromo-2, 2-dichloroethyl phosphate; molecular weight = 381) and permethrin ([3-Phenoxyphenyl] methyl-[+]-cis-trans-3-[2, 2-dichloroethenyl]-2, 2-dimethylcyclopropane carboxylate; molecular weight = 391.3). Malathion and naled donated by the Division of Plant Industry (DPI) in Gainesville and DPI in Winter Haven, Florida, respectively. Permethrin was purchased from Chemserve (West Chester, Pennsylvania).

The Monroe County Mosquito Control District (MCMCD) applies naled, a second-generation organophosphate insecticide, by aircraft (DC-3 and helicopter) throughout the Florida Keys. Planes/helicopters fly at an altitude of 50 m (165 ft) with swath widths of 61 m (200 ft) (Hennessey et al. 1992). The field level of naled application by MCMCD is 0.08 kg (AI)/ha as a 4% mixture with No. 2 diesel fuel (vol/vol). Likewise, diesel fuel is frequently used by MCMCD in their ground based ULV fog mixtures of another organophosphate insecticide malathion and a pyrethriod insecticide permethrin, so this substance was used as the control whenever possible to best simulate actual application conditions. Acetone was used as an alternate control when No. 2 diesel proved to be toxic to a test species. No. 2 diesel fuel was obtained from various gasoline stations and acetone was purchased from K-mart, both in Alachua County.

Test butterfly species and test procedure. The gulf fritillary, *Agraulis vanillae,* and the zebra longwing, *Heliconius charitonius,* were used as the experimental nymphalid butterflies to evaluate toxicity to topically applied mosquito control insecticides. Both species share similar habitat with *A. troglodyta floridalis* throughout south Florida. Larvae of both species were reared from eggs obtained at the Sanibel-Captiva Conservation Foundation (SCCF) in Sanibel, Florida, and from females reared at the Boender Endangered Species Laboratory in Gainesville, Florida. Developing larvae were fed a daily diet of *Passiflora* spp. leaves. Specimens were reared in plastic cups. Fifth instars and adults of both species were tested for toxicity of malathion and permethrin. Larvae were tested with treatment levels in logarithmic steps by applying one microliter (µl) of malathion or permethrin solution to the dorsum of the thorax with a Hamilton dispenser (PB600-1) fitted with a 25-µl syringe. Upon application, treatment groups were returned to their respective containers, provided fresh food, and monitored for 24 hours. Adult treatments were given in the same way. Adults were marked on the wing with colored ink to indicate the dosage used and released into a screen-enclosed flight cage at the Boender Endangered Species Laboratory or SCCF. These adults were provided with live flowering plants as a nectar source.

The Atala hairstreak, *Eumaeus atala* Rober, was used as a surrogate lycaenid butterfly in tests with mosquito control chemicals. Although *E. atala* is classified as a threatened species, large populations of the butterfly exist in parts of Dade County. One such area is Fairchild Tropical Gardens in Coral Gables. Here the larvae are considered a major pest of ornamental cycads, especially *Zamia pumila.* Early instars were obtained with permission on several occasions and reared to 5th instar for testing with naled and permethrin with the same techniques as those for the nymphalids. After treatment, adults were placed in small indoor cages for the test period, with each treatment in a different, labeled cage. Experimental survivors were brought to the SCCF for use in their butterfly enclosure exhibit.

The long-tailed skipper, *Proteus urbanus* (L.), and the tropical checkered skipper, *Pygrus oileus* (L.), are two hesperid species common throughout the pinelands of southern Florida and the Lower Keys. These species were tested as surrogates to determine the potential toxicity of naled and malathion toward *Hesperia meskei.* Larvae and adults of *P. urbanus* were collected from areas throughout Gainesville, Florida, where its host, beggar's tick, *Desmodium* spp., occurs. Additional *P. urbanus* specimens were collected in

TABLE 1. Comparison of mean adult *Anaea troglodyta floridalis* & *Strymon acis bartrami* densities per hectare at all transects for 1997 (29 sampling dates)–1998 (34 samples).

Transect	*A. troglodyta floridalis* 1997	*A. troglodyta floridalis* 1998	*S. acis bartrami* 1997	*S. acis bartrami* 1998
Nature Conservancy	0.5	1.0	6.2	6.9
Lytton's Way	0.0	0.2	2.6	2.6
Watson's Blvd.	0.0	0.2	4.8	2.3
Blue Hole	0.0	0.2	3.6	2.4
Watson's Hammock	2.2	5.0	1.7	2.5
Key Deer Refuge North	0.3	0.6	5.0	4.6
Key Deer Refuge South	0.3	0.9	8.6	5.0
Coconut Palm	0.0	1.9	5.2	3.7
Ixora Drive	0.5	1.5	1.7	8.4
Long Pine Key, Gate 4	2.4	2.9	0.0	0.0

Alva, Florida at sites along State Route 78. *Pygrus oileus* larvae and adults were obtained from hollyhock in July and August 1998 from northeastern Newberry, Florida. The larvae of both species roll a silken tent on the leaves of their respective hosts, making them easily located. Larvae were tested by the above procedures. As with the lycaenids, adult hesperids were placed in individual cages after treatments.

Lethal dosage analysis. Determination of lethal dosage values for various surrogate species and stages of south Florida Lepidoptera were derived using an innovative experimental design created by Peter J. Eliazar (Department of Entomology and Nematology, University of Florida) (Eliazar 1992, Eliazar in press). Prior to each test, replicate larvae/adults were weighed to obtain an average instar/imago weight for each species before treatment. Mortality data were pooled for each species and stage tested, to provide a larger sample for analysis. Experimental LD_{50} values were then determined with a probit analysis program run through an Apple Macintosh Microsoft Excel Spreadsheet. This probit analysis program, created by Dr. James L. Nation (Department of Entomology and Nematology, University of Florida), was derived from the equations and discussion found in Finney (1964). Table values were taken from Busvine (1971). LD_{50} values of each species were divided by the average instar or adult weight of that species to derive an "LD_{50} per gram of body weight" value. For direct comparison of lethal dosages between larvae and adults, and for comparison of LD_{50} values for other lepidopteran species, the "percent volume-to-volume concentration per gram of body weight" value was converted into "micrograms of active ingredient per gram of body weight" (µg/g). The levels of active ingredient (AI) are 1510, 1186 and 26.9 micrograms per microliter of concentrate for naled, malathion and permethrin, respectively. See Eliazar (1992) and Salvato (1999) for a more detailed description of the testing protocol used in these experiments.

RESULTS

Adult survey results. A total of 131 adult *Anaea troglodyta floridalis* was marked and released during the survey period. Means per hectare at each of the ten transects for the 1997 portion ranged from 0.0 at four Big Pine locations (Lytton's Way, Watson's Blvd., residential sites, Blue Hole and Coconut Palm within the refuge) to 2.4 within the Everglades. The Watson's Hammock site maintained the highest density on Big Pine with 2.2 per hectare (Table 1). There was no significant difference (F-test, $p = 0.05$) in *A. troglodyta floridalis* density between the control sites. There were significant differences, however, between both these control sites and four treatment areas for 1997 (Tukey's test, $p = 0.05$), with lower butterfly density in these sprayed locations.

A total of 97 adult *A. troglodyta floridalis* was recorded on dates between 18 January and 29 August 1998 (Table 1). Means during this period ranged from 0.2 individuals per hectare at Lytton's Way to 5.0 at Watson's Hammock (Table 1). There was no significant difference between control transects (F-test, $p = 0.05$); however, there were differences between both controls and three of the treatment sites in 1998 (Tukey's test, $p = 0.05$).

A total of 407 *Strymon acis bartrami* adults was marked and released, 232 during the 1997 and 175 in the 1998 sampling periods. During the sampling dates of July and August 1997, far more individuals were observed than could be marked by a single surveyor; this combined with the few recaptures illustrates the large density of *S. acis bartrami* present. The 1997 sampling period revealed per-hectare population means ranging from 0.0 (at Long Pine Key) to 8.6 on the southern Key Deer Refuge transect (Table 1). This low *S. acis bartrami* density at Long Pine was significantly different from the Watson's Hammock transect (Tukey's-test, $p = 0.05$). Two other sites (Nature Conservancy and southern Key Deer Refuge, both are insecticide treated areas) were also shown to have a significant difference with both control transects, in these cases, however, butterfly density was much higher on the insecticide treated spots than controls.

Again in 1998, the low population for *S. acis bartrami* was at Long Pine Key where no *S. acis bartrami* were recorded, thus being significantly different from Big Pine Key control site at Watson's Hammock (Table 1). Ixora Drive had the highest density on Big Pine at 8.4; this site, as well as the Nature Conservancy site, were both significantly different in hairstreak density (higher) than either control.

TABLE 2. Comparison of mean larval *Anaea troglodyta floridalis* and *Strymon acis bartrami* densities per hectare at all transects for 1997 (29 sampling dates)–1998 (34 samples).

	A. troglodyta floridalis		*S. acis bartrami*	
Transect	1997	1998	1997	1998
Nature Conservancy	0.5	1.6	0.0	0.2
Lytton's Way	0.3	1.5	2.1	0.6
Watson's Blvd	0.0	0.3	0.7	0.2
Blue Hole	0.2	4.9	1.0	0.9
Watson's Hammock	0.0	3.7	0.5	2.2
Key Deer Refuge North	0.2	1.3	0.0	0.2
Key Deer Refuge South	0.7	1.3	0.5	0.3
Coconut Palm	0.7	4.1	1.2	1.0
Ixora Drive	0.2	3.1	0.4	1.2
Long Pine Key, Gate 4	0.0	1.9	0.0	0.0

Adult *Hesperia meskei* were observed during the course of this study, but not within the transects. These sightings, first reported by Dr. Thomas C. Emmel on Big Pine, represent the first documented observations of the rock-land grass skipper in more than twenty years. A female specimen was taken by Dr. Jaret C. Daniels and the author on Big Pine on 2 June 1998.

Larval survey results. *Anaea troglodyta floridalis* larvae became increasingly more common at all transects as the survey progressed. A total of 168 larvae of various stages were marked in the field, 145 of these on Big Pine Key. Sixteen were marked in 1997 (July–December), all at Big Pine locations (Table 2). Per hectare mean estimates for this sampling period ranged from 0.0 at three locations (Watson's Blvd., Watson's Hammock and Long Pine) to 0.7 at both the southern Key Deer Refuge and Coconut Palm transects (Table 2). The remaining 152 larvae were marked in 1998 (January–August). Densities ranged from 0.3/ha on the residential Watson's Blvd. to 4.9/ha at Blue Hole (Table 2). The highest larval density occurred on 18 May 1998 at Blue Hole, where 18 larvae were marked. As with the adults, larvae of the Bartram's hairstreak were plentiful on Big Pine during the summer of 1997, with a total of 37 larvae marked from July to December (Table 2). Lytton's Way maintained the highest per hectare density at 2.1, while Nature Conservancy, north Key Deer Refuge and Long Pine had none. However, in keeping with the decrease in adult density to mid-1998, the number of larvae also declined slightly to 31 for the 1998 portion of the census. Four locations supported no larval activity (Watson's Blvd., north–south Key Deer Refuge and Long Pine). The un-sprayed Watson's Hammock had the highest density for 1998 with 2.2/ha. No *S. acis bartrami* larvae were found at Long Pine Key.

There were no significant differences found in larval density between transects for *S. acis bartrami* larvae in either survey year. However, there were significant differences in 1998 for larvae of the Florida leafwing on four transects, with these higher densities on areas that are normally treated with chemicals. Extremely dry conditions dominated the final two thirds of 1998, this alleviated the need for insecticide applications.

LD_{50} results. Following the experimental design of Eliazar (1992), LD_{50} values obtained using probit analysis (Salvato 1999) were divided by the average weight for the instar/imago of each species to give a percent volume-to-volume concentration per gram of body weight value. These values were then multiplied by micrograms of active ingredient (AI) per microliter of liquid concentrate to give an LD_{50} value expressed as µg/AI per gram of body weight (Table 4). These treatments showed naled and permethrin to be the most toxic, with LD_{50} values of 1.0 g or less of AI per gram of body weight for the surrogate species tested. LD_{50} values of 48.1 g or less of AI per gram of body weight were found for surrogates treated with malathion.

DISCUSSION

Adverse effects of pesticides on non-target organisms depend not only on the concentration of chemicals applied, but also on the pesticides' persistence and availability to susceptible life stages of the organisms (Pierce et al. 1989). During these surveys, transects were established on Big Pine to assess not only potential differences in butterfly density between treated and untreated areas, but also to measure and correlate the potential differences between the impact of drift and contact from aerial and/or ground ultra-low-volume applications. If indeed upwards of 70–80% of aerially delivered insecticides are lost in the canopy, then the nature of that canopy, the needles of slash pine or the herbaceous layer itself; as a landing site for the spray droplets needed to be considered (Fairchild et al. 1987).

Although these butterfly species are multivoltine, their numbers experience their largest increase at the same time as the seasonal increase in the insecticide applications. The largest adult densities for *A. troglodyta floridalis* were in Watson's Hammock, the no-spray area, both years. Similarly, 2.7/ha was the mean density for *A. troglodyta floridalis* in the Everglades. Chemical insecticide applications, which had formerly involved resmethrin by airplane, have not been performed at this location in 30 years. Thus these sites were considered control areas for comparison with those on Big Pine that are currently treated. These remaining insecticide treated transects (N = 8) were further divided by the insecticide applications

TABLE 3. Type of mosquito control application with influence to each transect and mean adult densities (per hectare) for *A. troglodyta floridalis, S. acis bartrami* and *H. meskei* over 63 sampling dates during 1997–98 on Big Pine Key and Everglades National Park.

Transect	Aerial spray	Ground spray	*A. troglodyta floridalis*	*S. acis bartrami*	*H. meskei*
Nature Conservancy	X	—	0.8	6.6	0.0
Lytton's Way	X	X	0.1	2.6	0.0
Watson's Blvd.	—	X	0.1	3.5	0.0
Blue Hole	X	X	0.1	2.9	0.0
Watson's Hammock	—	—	3.7	2.1	0.0
NKDWR (N)	—	X	0.5	4.8	0.0
NKDWR (S)	—	X	0.6	6.7	0.0
Coconut-Palm	X	X	1.0	4.4	0.0
Ixora Drive	—	X	1.0	5.3	0.0
Gate 4 at LPK	—	—	2.7	0.0	0.0

that might impinge upon them (aerial swath of ultra-low-volume or thermal naled fog, ultra-low-volume spray of truck applied thermal permethrin fog, or both). A comparison of all three treatment scenarios revealed no significant difference in adult butterfly mean densities over the survey period as a result of application type (Table 3). In all cases, *A. troglodyta floridalis* density failed to exceed 1.9/ha (Coconut Palm transect 1998) during this study, on chemically treated transects. *Anaea troglodyta floridalis* would appear at greater risk overall from both types of application, due to the fact that it flies at all levels of the canopy, including perching high atop the slash pines.

All surrogate nymphalid species tested proved very sensitive to chemical insecticides applied for mosquito control. *Heliconius charitonius* was found to possess a high sensitivity towards permethrin with LD_{50} values of 0.002 and 0.0004 µg of AI per gram of body weight for the fifth-instar and adult, respectively (Table 4). This species appeared less sensitive to malathion. It must be noted that all values provided for *H. charitonius* indicate the species toxicity solely towards the insecticide. These studies, as with those of Eliazar (1992) found a 100% mortality rate for *H. charitonius* towards the No. 2 diesel fuel. Because of this toxicity to No. 2 diesel fuel, acetone was used as the control substance for treatments using this species. However, No. 2 diesel fuel appeared to have a less dramatic effect on the other nymphalid tested, *Agraulis vanillae*. When tested with malathion, *A. vanillae* indicated LD_{50} values of 6.7 and 8.5 µg of AI per gram of body weight for fifth instar and adult, respectively (Table 4). These malathion values, while not as low as those recorded for this species towards naled (from Eliazar 1992) and permethrin, can still be classified as extremely toxic.

Immature and adult Atala hairstreaks were found to be equally susceptible to truck applied ULV permethrin as well as the aerially applied naled. Given the extreme sensitivity of this lycaenid to both pyrethroid and organophosphate insecticides, it is likely that threatened species, such as *Strymon acis bartrami,* are at grave risk to these applications. Due to the fact that this species commonly aggregates on the low-lying shrubs along disturbed roadsides, areas frequently targeted by ULV truck spraying, this butterfly would appear most at risk to insecticide applications of this type. If this is truly the case, then the potential *S. acis bartrami* densities indicated in this survey, especially during the summer 1997 sampling dates, are only a fraction of what might have been attained.

TABLE 4. LD_{50} values (in micrograms of active ingredient per gram of body weight) for all surrogate test species.

Species/Stage	Naled (acetone)	Naled (diesel)	Malathion	Permethrin
Proteus urbanus				
3rd instar	0.0699	—	0.2603	—
4th instar	0.0439	—	8.912	—
5th instar	0.0296	0.0889	0.3045	—
adult	0.1892	0.3632	13.458	—
Pygrus oileus				
4th instar	1.021	—	—	—
5th instar	0.304	—	—	—
adult	0.0823	—	—	—
Eumaeus atala				
5th instar	—	0.0009	—	0.0009
adult	—	0.0012	—	0.0036
Agraulis vanillae				
5th instar	—	—	6.572	—
adult	—	—	8.515	—
Heliconius charitonius				
5th instar	—	—	8.127	0.0015
adult	—	—	48.087	0.0004

The lower density of *S. acis bartrami* within the non-chemically treated areas appears to be in response to an inconsistent burn agenda and a negative effect on host density due to this fire restriction. Bartram's hairstreak larvae are dependent upon fresh host growth for development, *Croton linearis* is the single host both for this butterfly and *A. troglodyta floridalis.* The adult hairstreaks appear to require consistently occurring host for larger densities and successful dispersal (Salvato 1999, in press). Neither of these requirements seems to be met within the areas of Watson's Hammock or Long Pine Key used for this study.

Hesperiid species maintain populations in virtually every ecosystem type found throughout the keys. Both hesperiid species tested showed the greatest susceptibility to naled, followed by the ULV malathion. Large densities of *Aristida purpurascens* are also located where they are directly threatened by ULV truck applications, and other large populations of the plant exist across the open grass savannah areas of Big Pine within the Refuge. These large clumps of *A. purpuras-*

cens have allowed *H. meskei* to be perhaps the most exposed butterfly of the three rock-pineland occurring species discussed here to the full array of insecticides used for mosquito control. *Proteus urbanus* was found to be extremely sensitive to naled, in both juvenile and adult stages. The area in which *Hesperia meskei* were located during this survey was one not frequented by ULV trucks, but one that was still likely exposed to the aerial applications. The drier conditions on Big Pine during the last eight months of 1998 resulted in fewer mosquito control insecticide applications. The new observations of adult *H. meskei* and the enormous increases in *A. troglodyta floridalis* larval and adult activity in normally treated areas could likely be the result of this reduction in chemical spraying. Contrarily, the dry conditions had a negative impact on *Croton linearis* blooms and density, which directly affected *S. acis bartrami*, over a time frame apparently so favorable to the other two butterflies (Salvato 1999, in press).

The toxicity of permethrin to *E. atala* and *H. charitonius* indicated in this study may be underestimates. Permethrin was prepared in an non-synergized form. Further studies with this insecticide and these butterflies will need to include piperonyl butoxide (PBO), the synergist added to most pyrethroid insecticides to increase the potency and residual effects and thus likely their toxicity to non-target organisms.

According to Matsumura (1990), an insecticide that produces an LD_{50} value that is less than 1 μg/g of body weight is commonly classified as extremely toxic; 1–50 μg/g is highly toxic; 50–500 μg/g is considered moderately toxic and a value between 500–5000 μg/g is only slightly toxic. Beyond this last level, the chemical is considered practically nontoxic to relatively harmless. In regard to the Florida leafwing, Bartram's hairstreak, and rock-land grass skipper, the chemicals currently being applied for mosquito control in the Lower Florida Keys can be considered extremely to highly toxic, depending on the surrogate species and stage tested. Given the results of the Hennessey et al. (1991, 1992), insecticide drift experiments in Watson's Hammock, it is likely that chemical applications play an important role in affecting the population size and behavior of these species.

ACKNOWLEDGMENTS

The author thanks T. C. Emmel and J. L. Nation (Department of Entomology and Nematology, University of Florida) for advice and review of this manuscript, P. J. Eliazar for his many hours of instruction and assistance in determining the lethal dosage values presented, D. Serage (Sanibel-Captiva Conservation Foundation) for rearing and testing facilities, Fairchild Tropical Gardens for *E. atala* larvae. The author thanks J. C. Daniels, J. H. Frank, D. M. Griffin III, K. A. Schwarz, R. A. Worth, M. K. Hennessey and D. H. Habeck for their encouragement, suggestions and insights on this research, and the natural history of these organisms. The author would also like to thank all at National Key Deer Wildlife Refuge, Nature Conservancy and Everglades National Park for permitting and various technical support.

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Received for publication 8 November 1999; revised and accepted 19 July 2001.

ERRATA

INFLUENCE OF MOSQUITO CONTROL CHEMICALS ON BUTTERFLIES (NYMPHALIDAE, LYCAENIDAE, HESPERIIDAE) OF THE LOWER FLORIDA KEYS

In the above paper by Mark H. Salvato (Journal of the Lepidopterists' Society 55(1):8–14), there were seven typographical errrors:

pg. 10: *Proteus urbanus* (L.) should read *Urbanus proteus* (L.), *P. urbanus* should read *U. proteus*, *Pygrus oileus*(L.) should read *Pyrgus oileus* (L.).

pg. 11: *Pygrus oileus*(L.) should read *Pyrgus oileus* (L.).

pg. 13, Table 4: *Proteus urbanus* should read *Urbanus proteus*, *Pygrus oileus* should read *Pyrgus oileus*.

pg. 14: *Proteus urbanus* should read *Urbanus proteus*.