Susceptibility of *Culex pipiens* (Diptera: Culicidae) Field Populations in Cyprus to Conventional Organic Insecticides, *Bacillus thuringiensis* subsp. *israelensis*, and Methoprene

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ABSTRACT *Culex pipiens pipiens* L. populations on Cyprus were sampled over a 6-yr period from 2002 to 2008 to evaluate the status of insecticide resistance toward the insecticides temephos, chlorpyrifos, and permethrin and to study susceptibility levels toward the recently introduced bacterial insecticide *Bacillus thuringiensis* subsp. *israelensis* De Barjac and the juvenile hormone analog, methoprene. Susceptibility to the three conventional chemical insecticides varied between different collections, with most collections showing moderate or low resistance. The 2004 Akrotiri collection had the highest temephos resistance ratio, 167-fold at the $LC_{95,}$ although later sampling showed that the population returned to susceptibility after treatments stopped. Chlorpyrifos resistance was generally higher than temephos resistance. Four collections showed high resistance, and the resistance ratios of two collections were notably high with resistance ratios of 110- and 248-fold at the LC_{95}. Three collections showed high permethrin resistance (22.5-, 23.9-, and 86.3-fold). The frequency of elevated esterase activity in populations was estimated using a filter paper test, and frequencies varied from 0.9 to 65% among collections. The levels of temephos resistance and the frequency of elevated esterases in this survey were generally lower than in earlier reports, suggesting a decline in temephos resistance. Dose–response values for *B. thuringiensis* subsp. *israelensis* covered an approximate eight-fold range, but no resistance was detected. Methoprene values showed a 4.7-fold and 16-fold range at the LC_{50} and LC_{95}, respectively. Two populations showed significant resistance ratios at the LC_{95}. These data are discussed in relation to the changes in larval control practices underway in Cyprus.

KEY WORDS *Culex pipiens*, organophosphate resistance, *Bacillus thuringiensis* subsp. *israelensis*, methoprene

Cyprus has maintained an intensive program of mosquito surveillance and abatement since 1949, when malaria and the principle vector, *Anopheles sacharovi* (Favre), were eradicated from the island. The mosquito control program initially relied on DDT for larval control, but DDT was phased out in 1971 and replaced with temephos. In 1987, high levels of organophosphate (OP) resistance were detected in a population of *Culex pipiens pipiens* L., and a more extensive survey showed that OP resistance was widespread in *Cx. pipiens* populations (Wirth and Georghiou 1996). OP resistance was linked with two principle mechanisms: detoxifying enzymes and an insensitive target site (Wirth 1998). The detoxification enzymes consisted of four specific amplified esterases linked to organophosphate resistance: esterases A2, A5, B2, and B5. In addition, insensitive acetylcholinesterase was identified using a biochemical assay that measured enzyme activity in the presence and absence of paraoxon (Wirth 1998).

The high levels of OP resistance reported previously, in combination with concerns for environmental contamination, led to a ban on the importation of temephos to Cyprus in 2006 and necessitated a transition from traditional chemical insecticides to alternative, environmentally safe insecticides such as the bacterial insecticide, *Bacillus thuringiensis* subsp. *israelensis* (*Bti*), and the juvenile hormone analog, methoprene. In addition, the Ministry of Health established a Medical Entomology Laboratory under the Medical and Public Health Services. The Laboratory was charged with monitoring the status of OP resistance in mosquitoes, as well as testing and evaluating the efficacy of alternative insecticides to promote the transition to the new technologies.

Here we report on the results of recent field surveillance for susceptibility to insecticides in *Cx. pipiens.* In addition, the frequency of elevated esterase activity in

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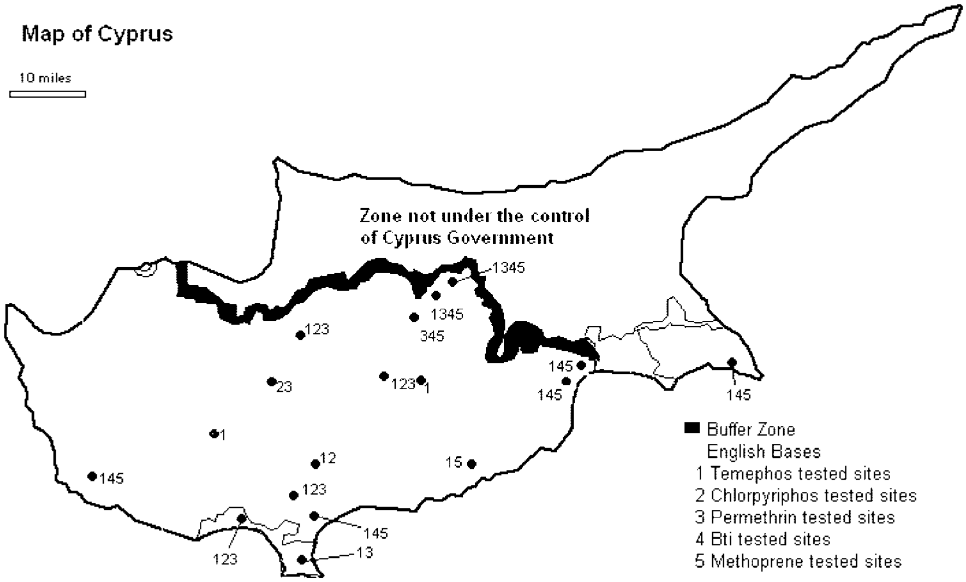

Fig. 1. Map of Cyprus showing the locations from which samples were collected for bioassay from 2002 to 2008. (1) Temephos-tested sites. (2) Chlorpyrifos-tested sites. (3) Permethrin-tested sites. (4) *Bti*-tested sites. (5) Methoprene-tested sites.

populations from different locations in Cyprus was estimated. Bioassays were also performed to establish the natural levels of susceptibility to the bacterial insecticide *Bti* and the juvenile hormone analog methoprene and to detect any significant changes in susceptibility.

Materials and Methods

Mosquito Collections. *Culex pipiens* larvae were collected from larval breeding sites at different locations in Cyprus from 2002 to 2008 (Fig. 1). Larvae were brought to the Medical Entomology Laboratory, identified to species (Darsie and Samanidou-Voyadjoglou 1997), and reared to adulthood. Larvae were fed a suspension of finely ground dog chow and brewers yeast (3:1). Adults were provided blood from chickens for egg raft production. The *Culex quinquefasciatus* Say laboratory colony, Slab, was obtained from the University of Montpellier II, Montpellier, France, to serve as a susceptible reference colony (Georghiou et al. 1966).

Insecticides. Formulated insecticides were used for most tests. The conventional insecticides included temephos (Abate, 50% [AI]; Spyros Stavrinides Chemicals, Nicosia, Cyprus), chlorpyrifos (technical material 98% [AI]; D. Shukuroglou, Latsia, Cyprus), and permethrin (Pounce, 38.47%; Spyros Stavrinides Chemicals). VectoBac SL (1,200 ITU/mg *Bti*, aqueous suspension; Valent Biosciences, Libertyville, IL), and Altosid (methoprene, 5% aqueous suspension; Omni-Chem, Louvain-la-Neuve, Belgium) were also tested.

Bioassay Tests. Initially, bioassays were conducted after collections were reared for an undefined period (within 6 mo of colonization) in laboratory culture. However, after 2004, all tests were performed on F_1, F_2, or F_3 generations. Bioassays used 20–25 early fourth instars placed in 100 ml distilled water in 150-ml glass cups. Conventional insecticide stock solutions were prepared in acetone. One milliliter of insecticide in acetone at the appropriate concentration was added to each cup, and mortality was evaluated after 24 h. Five to seven different concentrations were used in each test. A control with acetone without insecticide was prepared for each replication. *Bti* assays were similar to those for conventional insecticides except stock suspensions and controls were prepared with distilled water. Methoprene stocks were also prepared in distilled water, and exposed 100 late fourth instars to different concentrations for 24 h. Pupae that formed in 24 h were counted, removed to clean water, and allowed to emerge in a cage. The number of dead and/or unsuccessfully emerged individuals was counted. A minimum of four to six replications on different days was prepared for all tests.

Data were analyzed by Probit using SAS. Dose–response values for temephos and *Bti* were compared with values for Slab. However, no locally produced Slab assay data were available for the other insecticides; therefore, lethal concentration values for chlorpyrifos, permethrin, and methoprene were compared with unpublished data for Slab from the University of California, Riverside. Resistance ratios were calculated at the LC_{50} and LC_{95} by dividing the LC_{50} (or LC_{95}) value by the concurrent value obtained for Slab. For this study, resistance ratios <5 were not considered biologically significant, ratios of 5- to 20-fold were classified as moderately resistant, and ratios >20-fold were classified as highly resistant.

Filter Paper Test. The filter paper test for detecting esterase activity followed the procedure of Pasteur and Georghiou (1989) with minor changes. Briefly, individ-

Table 1. Dose–response values and estimated levels of resistance toward conventional organic insecticides in *Cx. pipiens* L. collected in Cyprus from 2002 to 2008

Insecticide	Collection site	Year	LC_{50} (FL) (μg/ml)	LC_{95} (FL) (μg/ml)	Resistance ratio	
					LC_{50}	LC_{95}
Temephos	Slab	2006	0.0116 (0.00865–0.0155)	0.0204 (0.0113–0.0385)	1.0	1.0
	Alampra	2002	0.00076 (0.00057–0.00101)	0.00210 (0.00115–0.00406)	<1.0	<1.0
	Agros	2003	0.0913 (0.0444–0.192)	0.257 (0.0248–3.03)	7.9	12.6
	Peristerona	2003	0.00389 (0.00346–0.00437)	0.0267 (0.0215–0.0349)	1.0	1.3
	Episkopi	2003	0.0500 (0.0350–0.0710)	0.163 (0.100–0.686)	4.3	8.0
	Kato Platres	2003	0.00421 (0.00205–0.00867)	0.216 (0.0273–1.73)	1.0	10.5
	Akrotiri	2004	0.310 (0.220–0.439)	3.40 (1.94–8.17)	26.7	167
	Marki	2004	0.0232 (0.0160–0.0338)	0.141 (0.0569–0.355)	2.0	6.9
	Agia Eirini	2004	0.0130 (0.00960–0.0177)	0.0488 (0.0262–0.0922)	1.1	2.4
	Nicosia (MEA)	2005	0.0144 (0.0134–0.0154)	0.0596 (0.0514–0.0714)	1.2	2.9
	Nicosia (KOA)	2005	0.0220 (0.0140–0.0310)	0.0860 (0.053–0.229)	1.9	4.2
	Nicosia (Platia Eleutherias)	2006	0.00831 (0.00425–0.0162)	0.0557 (0.0161–0.195)	1.0	2.7
	Pyla (Verki)	2006	0.00678 (0.00447–0.0104)	0.0381 (0.0136–0.114)	1.0	1.9
	Anarita	2006	0.0199 (0.00696–0.0563)	0.159 (0.0447–0.574)	1.7	7.8
	Akrotiri	2006	0.00858 (0.00755–0.00952)	0.0318 (0.0263–0.0418)	1.0	1.6
	Larnaka (General Hospital)	2006	0.0180 (0.0100–0.0460)	0.137 (0.0500–0.558)	1.6	6.7
	Ayia Napa	2007	0.00153 (0.00089–0.00259)	0.0247 (0.00971–0.0647)	1.0	1.2
	Lemesos	2008	0.00094 (0.00048–0.00186)	0.0119 (0.00223–0.0669)	1.0	1.0
	Nicosia (Yiorkio)	2008	0.00143 (0.00120–0.00168)	0.0118 (0.00897–0.0166)	1.0	1.0
	Kofinou	2008	0.00078 (0.00043–0.00140)	0.00745 (0.00223–0.0273)	1.0	1.0
Chlorpyrifos	Slab[a]	2003	0.00206 (0.00185–0.00230)	0.00310 (0.00244–0.00395)	1.0	1.0[a]
	Marki	2003	0.00320 (0.00280–0.00365)	0.0194 (0.0153–0.0259)	1.6	6.3
	Peristerona	2003	0.00354 (0.00202–0.00618)	0.0229 (0.00680–0.0782)	1.7	7.4
	Platanistasa	2003	0.00829 (0.00636–0.0108)	0.0765 (0.0445–0.134)	4.0	24.6
	Agros	2003	0.0520 (0.0343–0.0795)	0.769 (0.264–2.36)	25.2	248
	Agia Eirini	2004	0.0144 (0.0108–0.0194)	0.0626 (0.0343–0.116)	7.0	20.2
	Episkopi	2004	0.0726 (0.0464–0.114)	0.341 (0.107–1.11)	22.7	110
Permethrin	Slab[a]	2003	0.00088 (0.00078–0.00102)	0.0019 (0.0012–0.0021)	1.0	1.0
	Nicosia (Yiorkio)	2003	0.00151 (0.00151–0.00223)	0.00731 (0.00345–0.0368)	1.7	3.8
	Peristerona	2003	0.00205 (0.00179–0.00236)	0.0208 (0.0155–0.0303)	2.3	10.9
	Strovolos	2003	0.00252 (0.00171–0.00371)	0.0206 (0.00792–0.0549)	2.9	10.8
	Platanistasa	2003	0.00339 (0.00299–0.00385)	0.0248 (0.0186–0.0362)	3.8	13.0
	Akrotiri	2003	0.0363 (0.0316–0.0420)	0.0428 (0.312–0.636)	41.3	22.5
	Agia Eirini	2004	0.00346 (0.00307–0.00389)	0.0239 (0.0189–0.0321)	3.9	12.6
	Episkopi	2004	0.0475 (0.0330–0.0680)	0.164 (0.0801–0.356)	53.9	86.3
	Marki	2004	0.0105 (0.00950–0.0117)	0.0454 (0.0366–0.0599)	11.9	23.9

[a] Unpublished data from University of California, Riverside.

ual adult mosquitoes were homogenized in buffer (0.1 M $NaHPO_4$, pH 6.5, 0.5% Triton X-100), and 2 μl from each individual adult was deposited on a 1 by 1-cm piece of Whatman no. 2 filter paper. The filter paper strip was immersed in 5 ml of 0.1 M $NaHPO_4$ buffer with 0.1% α-naphthyl acetate for 60 s, blotted, transferred to 5 ml of 1.5 mg/ml Fast Garnet GBC salt in 0.1 M $NaHPO_4$ buffer for 60 s, blotted, briefly transferred to clean water, and air dried. The absorbance of the spots was read on a RCP Portable Color Reflective Densitometer (Tobias Associates, Ivyland, PA) using the red filter. Sixty or more individuals from 10 collections were analyzed. OD values greater than the mean of the susceptible Slab colony plus 2 SD (OD values > 0.55) were considered significantly higher than the susceptible reference population.

Results

A total of 19 collections were tested with temephos from 2002 to 2008. Their collection sites are shown in Fig. 1, and their dose–response values and resistance ratios are presented in Table 1. Eleven populations were not significantly different in susceptibility from the susceptible reference colony, Slab (based on overlapping fiducial limits), including Alampra (LC_{50} = 0.00076 μg/ml), Peristerona (LC_{50} = 0.00389 μg/ml), Agia Eirini (LC_{50} = 0.0130 μg/ml), Nicosia (MEA; 0.0144 μg/ml), Nicosia (Plateia Eleutherias; LC_{50} = 0.00831 μg/ml), Pyla (Verki; LC_{50} = 0.00678 μg/ml), Akrotiri (2006; LC_{50} = 0.00858 μg/ml), Ayia Napa (LC_{50} = 0.00153 μg/ml), Lemesos (LC_{50} = 0.00094 μg/ml), Nicosia (Yiorkio; LC_{50} = 0.00143 μg/ml), and Kofinou (LC_{50} = 0.00078 μg/ml). One collection, Nicosia (KOA), was significantly different from Slab but was not classified as resistant because its resistance ratio was less than five-fold. Six collections showed resistance ratios in the 5- to 20-fold range at either the LC_{50} or LC_{95} and were classified as moderately resistant, including Agros (RR_{95} = 12.6), Episkopi (RR_{95} = 8.0) Kato Platres (RR_{95} = 10.5), Marki (RR_{95} = 6.9), Anarita (2006; RR_{95} = 7.8), and Larnaka (General Hospital; RR_{95} = 6.7). The collection from Akrotiri showed resistance ratios of 26.7 and 167 at the LC_{50} and LC_{95}, respectively, in 2004 and was classified as highly resistant. However, another sample from Akrotiri was collected and tested in 2006 and was classified as susceptible.

Table 2. Dose–response values toward *B. thuringiensis israelensis* (*Bti*) and methoprene in *Cx. pipiens* L. collected in Cyprus from 2005 to 2008

Insecticide	Collection site	Year	LC_{50} (FL) (μg/ml)	LC_{95} (FL) (μg/ml)	Resistance ratio	
					LC_{50}	LC_{95}
Bti	Slab	2006	0.00256 (0.00176–0.00333)	0.00468 (0.00364–0.00803)	1.0	1.0
	Nicosia (MEA)	2005	0.00104 (0.00096–0.00138)	0.00280 (0.00237–0.00351)	1.0	1.0
	Nicosia (Platia Eleutherias)	2006	0.00155 (0.00138–0.00174)	0.00468 (0.00400–0.00609)	1.0	1.0
	Nicosia (KOA)	2006	0.00128 (0.0111–0.0153)	0.00176 (0.00493–0.0153)	1.0	1.0
	Anarita	2006	0.00167 (0.00157–0.00180)	0.00571 (0.00485–0.00701)	1.0	1.2
	Larnaka (General Hospital)	2006	0.00345 (0.00245–0.00507)	0.00775 (0.00585–0.0124)	1.3	1.7
	Pyla (Verki)	2006	0.00105 (0.00087–0.00127)	0.00468 (0.00308–0.00658)	1.0	1.0
	Ayia Napa	2007	0.00129 (0.00111–0.00148)	0.00786 (0.00636–0.0102)	1.0	1.7
	Lemesos	2008	0.00078 (0.00066–0.00092)	0.00681 (0.00497–0.0103)	1.0	1.5
	Kofinou	2008	0.00124 (0.00107–0.00143)	0.00713 (0.00559–0.00973)	1.0	1.5
	Nicosia (Yiorkio)	2008	0.00205 (0.00146–0.00286)	0.0141 (0.00698–0.0305)	1.0	3.0
Methoprene	Slab[a]	2000	0.00144 (0.00105–0.00197)	0.00509 (0.00229–0.00116)	1.0	1.0
	Nicosia (MEA)	2005	0.00287 (0.000126–0.00407)	0.0374 (0.0180–0.358)	2.0	7.3
	Nicosia (Platia Eeutherias)	2006	0.00610 (0.00049–0.00075)	0.00398 (0.00292–0.00593)	1.0	1.0
	Larnaka (General Hospital)	2006	0.00122 (0.000096–0.00147)	0.00757 (0.00579–0.0112)	1.0	1.5
	Ayia Napa	2007	0.00171 (0.00141–0.00207)	0.0436 (0.0299–0.0691)	1.2	8.6
	Lemesos	2008	0.00208 (0.00165–0.00261)	0.0641 (0.0389–0.124)	1.4	12.6
	Nicosia (Yiorkio)	2008	0.00199 (0.00150–0.00264)	0.0314 (0.0185–0.0547)	1.4	6.2
	Kofinou	2008	0.00113 (0.00093–0.00135)	0.0219 (0.0161–0.0317)	1.0	4.3

[a] Unpublished data from UC Riverside.

Six collections were tested in 2003–2004 with chlorpyrifos and compared with unpublished Slab data from UC Riverside. The Peristerona and Marki collections showed moderate resistance at the LC_{95} with values of 0.0194 and 0.0229 μg/ml and resistance ratios of 7.4 and 6.3, respectively. The remaining four collections showed high levels of chlorpyrifos resistance at LC_{95}. Platanistasa had an LC_{95} of 0.0765 μg/ml and a resistance ratio of 24.6, whereas Agia Eirini had an LC_{95} of 0.0626 μg/ml and a resistance ratio of 20.2. Agros and Episkopi showed the highest observed levels of chlorpyrifos resistance. Agros showed an LC_{50} of 0.0520 μg/ml and an LC_{95} of 0.769 μg/ml, with resistance ratios of 25.2 and 248, respectively. Episkopi had LC_{50} and LC_{95} values of 0.0726 and 0.341 μg/ml and resistance ratios of 22.7 and 110, respectively.

Eight collections were tested with permethrin in 2003–2004 and compared with unpublished Slab data from UC Riverside. One collection, Nicosia (Yiorkio) showed no significant resistance, with an LC_{50} of 0.00151 μg/ml and an LC_{95} of 0.00731 μg/ml. Resistance ratios were 1.7 and 3.8, respectively. However, the remaining seven collections showed moderate to high permethrin resistance. Peristerona, Strovolos, Platanistasa, and Agia Eirini showed moderate resistance with resistance ratios at the LC_{95} of 10.9, 10.8, 13.0, and 12.6, respectively. Three collections had high resistance. Akrotiri showed 22.5-fold resistance at the LC_{95} and Marki showed 23.9-fold resistance at the LC_{95}. The highest level of resistance was observed in the Episkopi collection. Episkopi had an LC_{50} of 0.0475 μg/ml and an LC_{95} of 0.164 μg/ml. Resistance ratios were 53.9 at the LC_{50} and 86.3 at the LC_{95}.

Ten collections were assayed with *Bti* from 2005 to 2008, and the lethal concentration values were compared with the susceptible *Cx. quinquefasciatus* reference colony Slab (Table 2). Two collections, Nicosia (MEA) and Nicosia (KOA), were significantly more susceptible than Slab at both the LC_{50} and LC_{95}. Nicosia (Platia Eleutherias), Pyla (Verki), Ayia Napa, Lemesos, and Kofinou were significantly more susceptible than Slab at the LC_{50} but not at the LC_{95}. Anarita, Larnaka (General Hospital), and Nicosia (Yiorkio) were not significantly different in susceptibility from Slab. LC_{50}s ranged from 0.00104 to 0.00345 μg/ml, a 3.3-fold range, whereas the LC_{95}s ranged from 0.00176 to 0.0141 μg/ml, an 8-fold range.

Seven collections were assayed with methoprene from 2005 to 2008 and compared with values for Slab generated by UC Riverside. Nicosia (Plateia Eleutherias) was the most susceptible population, with LC_{50} and LC_{95} values of 0.000610 and 0.00398 μg/ml, respectively. Interestingly, an earlier sample from a different site in Nicosia (MEA) showed 7.3-fold resistance at the LC_{95}. Methoprene was continued in use over this period; therefore, the high value from 2005, which was not detected in the sample from 2006, may be because of technical issues, although the two samples were not from identical sites. Given this ambiguity, the 2005 data point can only be classified as questionable. Two other collections, Larnaca (General Hospital) and Ayia Napa, showed no significant changes in susceptibility, with LC_{50} values of 0.00122 and 0.00171 μg/ml. Two collections, Lemesos and Nicosia (Yiorkio), showed significantly elevated LC_{95} values of 0.0641 and 0.0314 μg/ml and resistance ratios of 12.6 and 6.2, respectively. Kofinou, with an LC_{95} of 0.00219 μg/ml, showed an elevated resistance ratio of 4.3-fold but was below the 5-fold significance threshold of the study. Overall, LC_{50} values varied 4.7-fold, whereas LC_{95} values varied 16-fold.

One hundred adult mosquitoes from Slab were analyzed with the filter paper test to establish a statistical distribution for that population to compare with the

Table 3. Statistical analysis of esterase activity distribution in adult *Cx. pipiens* from various locations in Cyprus, 2003–2004

Colony	Year	Mean OD value	SD	No. positive[a]/ no. tested	Percent positive
Slab	2006	0.4132	0.1203	NA	NA
Platanistasa	2003	0.4726	0.0684	16/216	7.4
Agia Erini	2003	0.4717	0.0905	37/168	22.0
Larnaka	2003	0.4820	0.0890	16/100	16.0
Agia Eirini	2004	0.4478	0.0530	1/108	0.9
Marki	2004	0.4739	0.0833	24/188	12.8
Akrotiri	2004	0.576	0.136	119/204	58.3
Kornos	2004	0.5683	0.1505	39/60	65

[a] OD values greater than the mean of Slab + 2 SD units (0.4132 + 0.2406) were considered positive for elevated esterase activity.
NA, not applicable.

Cypriot collections made in 2003–2004. OD readings ranged from 0.29 to 0.52, with a mean of 0.4132 (SD = 0.1203; Table 3). OD values greater than the mean of Slab plus 2 SD (0.56 or higher) were classified as having significantly elevated esterase activity. Seven collections were analyzed from 2003 to 2004, and the mean OD value and SD were calculated (Table 3). The frequency of elevated esterase activity ranged from a low of 0.9% for Agia Eirini to a high of 65% for Kornos. The majority of collections had elevated esterase frequencies between 8.9 and 16.0%. Two other collections showed distinctly higher frequencies; Akrotiri had a frequency 58.3% and Kornos had a frequency of 65%. The frequency distribution for the Kornos collection is shown (Fig. 2).

Discussion

Culex pipiens collected from 2002 to 2008 from various locations in Cyprus showed low to moderate levels of resistance toward temephos, with few exceptions. Akrotiri, with 167-fold resistance to temephos, was notable for its resistance level. Despite that exception, the remaining collections had resistance ratios of 10-fold or less, and 11 of 19 collections had <3-fold resistance to temephos at the LC_{95}. The resistance levels in this survey are lower than samples collected in 1993, in which all collections tested had >10-fold resistance to temephos (Wirth and Georghiou 1996). This may be because of the reduction in temephos exposure after the transition to alternative control materials. Some evidence supporting this hypothesis is found in the two Akrotiri collections. In 2004, the Akrotiri area was declared a protected area, and all pesticide use was banned. Resampling in 2006 indicated that temephos dose–response values had returned to susceptible levels. Other Mediterranean countries, including Italy (Silvestrini et al. 1998), Spain (Eritja and Chelvillon 1999), and Israel (Orshan et al. 2005), reported declines in OP resistance in *Culex* populations when the treatment insecticide was removed or replaced. The reductions were attributed to fitness costs linked with the resistance alleles (Berticat et al. 2008).

Overall, chlorpyrifos resistance was higher than temephos resistance, because four of six samples showed high resistance. Although the samples tested with chlorpyrifos were collected early in the study, from 2003 to 2004, it should be noted that temephos resistance ratios from 2003 to 2004 were considerably lower than those for chlorpyrifos. This suggests that chlorpyrifos resistance reached higher levels than did temephos and may continue to present some problems. Unlike temephos, chlorpyrifos resistance ratios were estimated using the unpublished Slab data from UC Riverside as the reference baseline and not by the preferred method of concurrent bioassays with the same technical material; therefore, this interpretation should be weighed cautiously.

Pyrethroid insecticides are used as larvicides in municipalities, as adulticides for home use, and widely in agriculture. Seven of the eight collections showed dose–response values that were statistically higher than the Slab data from UC Riverside. One collection, Episkopi, collected near an agricultural area, showed the highest level of pyrethroid resistance, with a resistance ratio of 86.3 at the LC_{95}. Permethrin resistance was reported in two of the six collections in the previous survey (Wirth and Georghiou 1996) compared with seven of eight collections in this study,

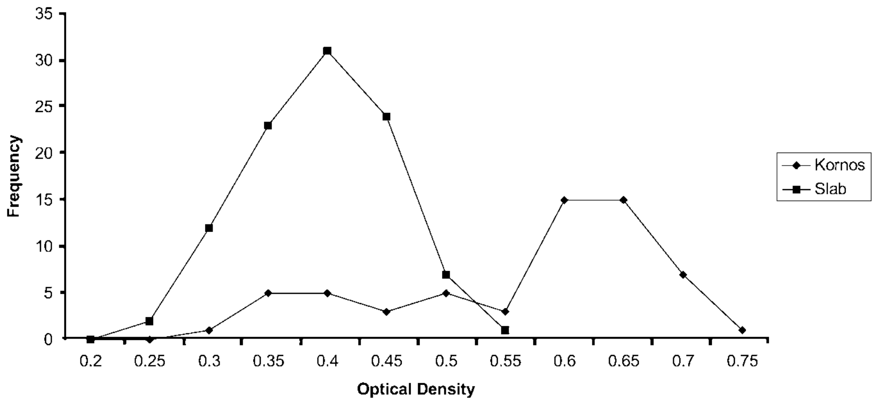

Fig. 2. Frequency distribution of esterase activity from the susceptible *Cx. quinquefasciatus* colony, Slab, and the collection of *Cx. pipiens* from Kornos measured using the filter paper assay.

suggesting an increase in pyrethroid resistance since that earlier study. Again, caution in interpreting these data is needed because of the source of the susceptible reference data. Pyrethroid resistance has been reported in Mediterranean populations of *C. pipiens* from Saudi Arabia (Amin and Hemingway 1989), Egypt (Zayed et al. 2006), and Tunisia (Daaboub et al. 2008). The mechanism of pyrethroid resistance in Cyprus populations has not been identified, but cytochrome P450 monooxygenase-mediated detoxification and *kdr* have been reported in some Mediterranean populations (Kasai et al. 1998, Daaboub et al. 2008).

Dose–response data using formulated *Bti* on 10 collections showed a homogeneous response among the collections. Slab, the *Cx. quinquefasciatus* laboratory susceptible colony, had the second highest LC_{50} value of 0.00256 μg/ml and was exceeded only by that of Larnaka (General Hospital) with an LC_{50} of 0.00345 μg/ml. Five collections showed LC_{95} values greater than Slab, whereas the remaining five collections were more susceptible. This most likely represents the broader population heterogeneity of the field collections relative to a highly homogeneous laboratory reference colony. Interestingly, *Bti* was previously tested against seven Cypriot collections in 1993. Those collections showed 6- to 11-fold variation in LC values (Wirth and Georghiou 1996), which is not that different from the 3- to 8-fold variation observed here. Unlike this study, the earlier tests relied on *Bti* (IPS 80) technical material and were conducted at a different laboratory—both are potential sources of variability. Although laboratory selection studies have shown very low risk for the evolution of *Bti* resistance in *Cx. pipiens quinquefaciatus* (Georghiou and Wirth 1997), a report of possible *Bti* resistance in *Cx. pipiens* from New York (Paul et al. 2005) raises concerns for its long-range utility and reinforces the need to regularly monitor population susceptibility.

Seven collections were successfully assayed with methoprene. Only Nicosia (Yiorkio) was not significantly different from Slab. The three populations showed significantly elevated LC_{95} values. Although some variation may be because of the inherent technical difficulties in assaying methoprene [such as the early Nicosia (MEA) dose–response line], these results raise concerns for the long-term reliability of methoprene for control and emphasize the importance of establishing integrated resistance management program for methoprene. Field resistance to methoprene was documented to evolve after 20 yr of use against *Ochlerotatus nigromaculus* (Ludlow) in central California (Cornel et al. 2002). Furthermore, laboratory selection studies have shown the potential of *Cx. pipiens pipiens* and *Culex tarsalis* (Coquillett) to evolve resistance to methoprene (Brown and Brown 1974, Georghiou 1974). Consequently, routine monitoring of methoprene susceptibility will be essential to determine its continuing usefulness.

The filter paper esterase tests gave a snapshot of the frequency of elevated esterase activity in the collections before the temephos ban was initiated. The test, although rapid and simple, does not identify the different esterase allozymes, nor does it provide information on the presence or absence of insensitive acetylcholinesterase. Both require equipment and technical skills that are beyond the capabilities of the laboratory at present. However, the test offers a simple, rapid method to monitor changes in the frequency of elevated esterase, which over time may prove informative as the transition away from conventional insecticides continues. It remains to be seen whether elevated esterase activity is retained in populations or declines and/or disappears in the continued absence of temephos selection pressure as reported in Italy (Silvestrini et al. 1998). Although temephos resistance levels declined in the Akrotiri collections after treatments ceased, it is not certain that elevated esterase frequencies will decline in all areas. Some OP resistance genes were reported to be stable in the absence of treatment in *Cx. pipiens* populations, presumably because of a weak OP resistance cost or indirect selection pressure from pesticide pollution (Eritja and Chevillon 1999).

The Ministry of Health and the Public Health Service of Cyprus have established a framework to support the transition from conventional synthetic organic insecticides to an integrated pest management (IPM) approach for controlling mosquito populations. That strategy includes incorporating environmentally safe insecticides such as *Bti* and methoprene and monitoring for changes in susceptibility of treated populations. The long-term benefits of this strategy are expected to include improved mosquito control, a reduction in the level of organic pesticides entering aquatic systems and the environment, a decline in the organophosphate resistance levels in mosquito populations, and an improved quality of life for residents and visitors.

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