BIOLOGICAL FITNESS OF A *CULEX QUINQUEFASCIATUS* POPULATION AND ITS RESISTANCE TO *BACILLUS SPHAERICUS*

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ABSTRACT. Biological fitness components of a field-collected colony of *Culex quinquefasciatus* Say that was highly resistant to *Bacillus sphaericus* strain 2362 (resistance ratio greater than 163,000) after 46 generations of selection were compared to those of a susceptible colony (CqSF) that had originated from the same parental cohort but that had not been exposed to *B. sphaericus*. The effect of *B. sphaericus* on the fitness of *Cx. quinquefasciatus* was determined in terms of fecundity, fertility, and development time. The resistant colony (CqRL) showed significantly lower fecundity and fertility, and slower development than the susceptible colony. Development time from egg to egg showed a 20% increase in CqRL compared to CqSF. The generation time increased from 21.6 days to 26 days for highly resistant generations of CqRL.

KEY WORDS   *Bacillus sphaericus*, *Culex quinquefasciatus*, fitness cost, resistance, laboratory selection

INTRODUCTION

Strains of *Bacillus sphaericus* with high toxicity to mosquito larvae have been used for the control of disease vectors. Larvicidal activity of *B. sphaericus* is caused by a parasporal crystal produced during sporulation. The crystal contains a binary toxin formed by polypeptides of 42 and 51 kDa, named BinA and BinB, respectively. The binary toxin complex acts by binding specifically to midgut receptors of mosquito larvae, as has been demonstrated in some susceptible mosquito species (Nielsen-LeRoux and Charles 1992, Silva-Filha et al. 1997).

In many areas of the world, resistance to conventional insecticides has increased interest in larvicides such as *B. sphaericus* (Georghiou and Lagunes-Tejeda 1991). Products based on *B. sphaericus* have been used successfully in large-scale field programs against *Culex* and *Anopheles* species in several countries. However, in recent years, resistance to *B. sphaericus* has been recorded in populations of *Culex* that have been subjected to strong selection pressure under laboratory conditions (Georghiou et al. 1992, Rodcharoen and Mulla 1994, Wirth et al. 2000), or when field populations were routinely treated (Sinègre et al. 1994, Adak et al. 1995, Rao et al. 1995, Silva-Filha et al. 1995, Yuan et al. 2000). Two different mechanisms of resistance have been reported; one is related to the loss of binding of the Bin toxin to the receptor and the other, which does not involve a change in the binding step, remains unknown (Nielsen-Le-Roux et al. 1997).

Several studies have been conducted on the biological fitness of resistant mosquitos to various synthetic insecticides (Ferrari and Georghiou 1981, Amin and White 1984, Bonning and Hemingway 1991). However, mosquito resistance to *B. sphaericus* is a relatively new occurrence and no studies

except those of Rodcharoen and Mulla (1997) have been conducted on biological fitness of *B. sphaericus*–resistant mosquitoes. As is the case in other insect groups, insecticide resistance in mosquito populations often is associated with lower fecundity and longer development time than in their susceptible counterparts (Georghiou and Taylor 1977); however, in many cases, the differences in biotic potential between resistant and susceptible insect strains are found to be small (Thomas and Brazzel 1961, Roush and Hoy 1981).

This study investigated some aspects of the biological fitness of 2 colonies of *Culex quinquefasciatus* Say—1 highly resistant (resistance ratio [RR] > 163,000) and 1 susceptible to *B. sphaericus*. The resistant colony was obtained under continuous laboratory selection with *B. sphaericus* strain 2362 for 46 generations (Pei et al. 2002).

MATERIALS AND METHODS

Mosquitoes: Two colonies of *Cx. quinquefasciatus* were used in this study. A *B. sphaericus*–susceptible strain (CqSF) originated from a large number of egg rafts collected from different breeding sites in Recife, Brazil, and was maintained in the laboratory for at least 3 years without exposure to *B. sphaericus*. The resistant colony (CqRL) was started from the CqSF colony and was subjected to continuous selection pressure with *B. sphaericus* strain 2362. The selection procedure consisted of exposing a large number (10,000–20,000) of young 4th-stage larvae to *B. sphaericus* at concentrations ranging from the median lethal concentration to the 93% lethal concentration for 46 generations, when an RR greater than 163,000 was obtained (Pei et al. 2002). Two generations of the CqRL colony were investigated in this study: a generation that displayed a moderate degree of resistance, generation F25 (RR = 64.05), which is referred to as

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Table 1. Reproductive potential and survival rates of colonies of *Culex quinquefasciatus* that were susceptible (CqSF), moderately resistant (CqRL-F25), and highly resistant (CqRL-F47) to *Bacillus sphaericus* strain 2362.

Stage and event	Susceptible	Resistant	
		F25	F47
No. eggs/raft (mean ± SD)	216.5 ± 20.6	218.7 ± 26.0	199.6 ± 30.3
No. L1/raft[1] (mean ± SD)	197.6 ± 40.3	160.3 ± 46.2	167.1 ± 31.5
Mean % hatch	91.2	73.3	83.7
No. adults/raft (mean ± SD)	189.2 ± 39.5	149.7 ± 43.6	155.1 ± 29.2
Mean % emergence[2]	95.7	93.4	92.8
Mean % adults[3]	87.4	68.4	77.7

[1] L1, 1st-stage larvae.
[2] Percentage of adults based on the number of L1.
[3] Percentage of adults based on the number of eggs.

CqRL-F25, and a later generation that displayed high resistance (RR > 163,000), which is referred to as CqRL-F47. The mosquitoes were reared at 27–28°C, 87% relative humidity, and 12:12 h light: dark photoperiod.

Fitness cost assessment: The parameters fecundity, fertility, preoviposition period, development time, preadult survivorship, and generation time were compared between the 2 colonies (CqSF and CqRL) to determine whether resistance to *B. sphaericus* was associated with any reproductive disadvantage.

Egg rafts were taken from female mosquitoes that had not been exposed to *B. sphaericus* during their larval stage. Fully bloodfed females were selected randomly from each CqSF and CqRL colony and allowed to lay eggs. Fecundity was then measured by using 20 egg rafts from each colony and determining the average number of eggs per raft at the 1st gonotrophic cycle.

Fertility was assessed as the mean number of 1st-stage larvae (L1) and the percentage of eggs that hatched within 24 and 48 h after oviposition. Twenty egg rafts were used from the CqSF and CqRL-F25 colonies and 10 were used from the CqRL-F47 colony. Each egg raft was placed individually in a plastic cup containing 200 ml of distilled water.

The preoviposition period was determined by recording the female imaginal age at the 1st oviposition. Fifty randomly selected, newly emerged and well-fed females were taken from each colony and allowed to lay eggs. Those that did not lay eggs until 5 days after the 1st blood meal were refed and followed until oviposition.

Preadult development time and survivorship were assessed by accompanying larvae from 10 egg rafts of each susceptible and resistant colony. Larvae from each egg raft were reared in a plastic pan filled with 1 liter of dechlorinated water and fed ground cat chow (Whiskas, Eldorado do Sul, Brazil). Larvae were counted every day until pupation. The pupae were transferred daily to a 200-ml cup and placed in screen cages for adult emergence.

Generation time (from egg to egg) was compared throughout a 3-year period of resistance selection, during which the RR of the CqRL colony increased

from 1 to 18 in the 1st year, to 137 in the 2nd year, and was greater than 163,000 at the end of the 3rd year of resistance selection.

RESULTS

Reproduction characteristics

No difference in fecundity ($P > 0.1$) was found between the susceptible (CqSF) and the moderately resistant (CqRL-F25) females. However, highly resistant females (CqRL-F47) produced significantly fewer eggs ($P < 0.0001$) at the 1st gonadotrophic cycle compared with the CqSF females (Table 1). All egg rafts produced by the resistant and susceptible females were viable, indicating that successful mating occurred in both groups. The hatching rate for both CqRL-F25 and CqRL-F47 was significantly lower ($P < 0.0001$) than in the susceptible CqSF colony (Table 1). First oviposition was delayed in the highly resistant CqRL-F47 as compared with the susceptible group ($P = 0.0001$); the highly resistant mosquitoes required 2 blood meals to mature and deposit their 1st egg raft, which occurred 12.7 days on average after adult emergence, whereas 41.4% of the susceptible CqSF females had laid their 1st batch of eggs 3–4 days after the 1st blood meal (Fig. 1 and Table 2).

Development time

Virtually all eggs (>99%) from the susceptible CqSF and the moderately resistant CqRL-F25 colonies hatched within 24 h after oviposition, whereas the eggs from the highly resistant group CqRL-F47 hatched between 24 and 48 h after oviposition. No difference was recorded in larval-pupal survivorship between the CqSF strain and the CqRL-F47 strain; however, a lower yield of adults per raft was noted in the resistant strains, as a result of lower fecundity, lower hatching rates, or both (Table 1). The majority of larvae pupated between the 8th and the 16th day in susceptible and highly resistant colonies and no statistical difference ($P = 0.207$) was found in the mean time from egg hatch to pupation between the groups (Table 2). However, the total
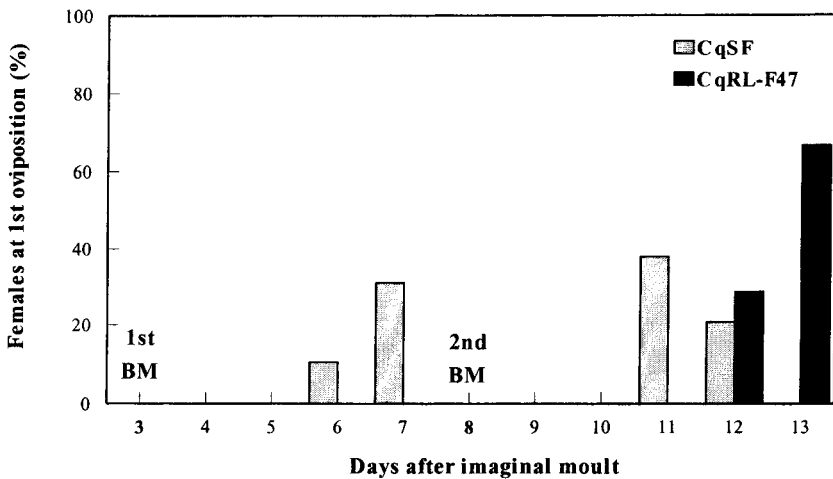
Fig. 1. Age at 1st oviposition of 50 female *Culex quiquefasciatus* from susceptible (CqSF) and highly resistant (CqRL-F47) colonies. BM, blood meal.

development time, recorded from egg to egg, was significantly different ($P = 0.0001$), ranging from 17 to 34 days for the susceptible and from 24 to 40 days for the highly resistant colony (Table 2). This difference was due to a longer embryonic development and delayed oviposition observed in the resistant group. Examination of data obtained from larger numbers of individuals during resistance selection (approximately 10,000 larvae exposed to *B. sphaericus* each generation) revealed that the generation time showed small variation throughout the 1st 2 years of selection, lasting 21.6 ± 3.2 days (16.8 generations per year). However, this increased to 26.2 ± 1.26 days (13.9 generations per year) during the last year of resistance selection.

DISCUSSION

Several differences in biological fitness were detected between colonies of *Cx. quinquefasciatus* susceptible and resistant to *B. sphaericus*. Both of the CqRL generations (F25 and F47) that were evaluated demonstrated a significantly reduced fertility compared to the CqSF. Fertility was lower, and as a consequence so was adult yield, in the CqRL-F25 generation compared to the CqRL-F47 gener-

ation. However, the latter also showed reduced fecundity. Reduced fecundity and fertility have been described previously in mosquito populations that became resistant to synthetic insecticides (Ferrari and Georghiou 1981, Amin and White 1984, Rowland 1991). More recently, Rodcharoen and Mulla (1997) demonstrated that strains of *Cx. quinquefasciatus* resistant to *B. sphaericus* showed significantly lower fecundity and fertility than their susceptible counterparts, concluding that the resistant strains exhibited fitness disadvantages in the absence of *B. sphaericus*.

In addition to the effects on egg production and hatching rates, females from the highly resistant generation (CqRL-F47) demonstrated delayed oviposition and their offspring demonstrated slower embryonic development. As a consequence of these factors, generation time increased, resulting in a decrease of approximately 3 generations per year as resistance progressed.

The difference in development and reproduction between the 2 colonies could be attributed to the resistance gene, or to gene(s) closely linked to the resistance locus, given that the colonies have similar genetic background, were derived from the

Table 2. Development time of colonies of *Culex quinquefasciatus* susceptible (CqSF) and highly resistant (CqRL-F47) to *Bacillus sphaericus* strain 2362, determined by using 10 egg rafts from each colony.

Mosquito strain	Development time (days)				
	From oviposition to egg hatching	L1[1] to pupa mean ± SD (minimum–maximum)	Pupa to adult	IM[2] to oviposition mean ± SD (minimum–maximum)	From egg to egg mean ± SD (minimum–maximum)
Susceptible	1	11.3 ± 2.1 (8–19)	2	9.4–2.3 (6–12)	23.7 ± 4.5 (17–34)
Resistant (F47)	2	10.5 ± 1.7 (8–23)	2	12.7 ± 0.4 (12–13)	27.2 ± 2.7 (24–40)

[1] L1, first-stage larva.
[2] Imaginal molt.

same field-collected sample, and were maintained under identical laboratory conditions. Resistant larvae used in fitness experiments were not exposed to *B. sphaericus* and females used to assess fecundity and fertility had not been exposed to *B. sphaericus* in their larval stage in order to avoid the effects of sublethal doses on adult reproduction. In addition, only those eggs harvested from the 1st gonadotrophic cycle were considered to avoid the female age effect on fecundity and fertility (Suleman and Reisen 1979, Amin and White 1984). However, it is difficult to discard completely any possible influence of direct or indirect cumulative effects of larvicide treatment throughout the selection process, for example, by causing a lower nutritional reserve in survivors at each generation. It is noteworthy that *B. sphaericus* acts as a per os larvicide and that the larval midgut is its 1st target site. Lacey et al. (1987) observed that regardless of the mechanism that enables larvae that have ingested the toxin to survive, the difference in stored nutrient reserves between treated survivors and control adults indicated a disruption of the full potential for acquiring available nutrients.

Examination of our data, which were obtained under controlled laboratory conditions, showed that a biological disadvantage develops along with the ability to survive treatment with *B. sphaericus*. Although it is difficult to apply these data to field situations, a fitness cost of resistance, albeit slight, associated with immigration of susceptible individuals may help the decline of the resistant population. Studies on resistance to *B. sphaericus* performed on different populations of *Culex pipiens* L. have demonstrated a recessive inheritance (Nielsen-LeRoux et al. 1997, Wirth et al. 2000). The immigration of homozygous susceptible genomes and the lower biological fitness of the resistant population serves to decrease the frequency of the resistance allele in the mosquito population. A rapid reversal of a high level of resistance to *B. sphaericus* was observed in a field population in China where, 6 months after stopping treatment, the resistance ratio to *B. sphaericus* had decreased from 22,672- to 5.78-fold (Yuan et al. 2000). In Recife, Brazil, a field population with a low level of resistance to *B. sphaericus* became completely susceptible 11 months after suspension of treatment (Silva-Filha and Regis 1997). In both cases, reversal of resistance was mainly attributed to the immigration of susceptible individuals from untreated surrounding areas or untreated refuges within the target area.

Mosquito populations are able to recover rapidly after suspension or decrease in control pressure and so the best measure to assure this pressure in control programs should be to prevent or delay the establishment of resistance. In this sense, the integration of the use of spraying of *B. sphaericus* with other environmentally safe agents, such as *Bacillus thuringiensis israelensis,* is highly recommended.

REFERENCES CITED

Adak T, Mittal PK, Raghavendra K. 1995. Resistance to *Bacillus sphaericus* in *Culex quinquefasciatus* Say 1823. *Curr Sci* 69:695–698.

Amin AM, White GB. 1984. Relative fitness of organophosphate-resistant and susceptible strains of *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bull Entomol Res* 74:591–598.

Bonning BC, Hemingway J. 1991. Identification of reduced fitness associated with an insecticide resistance gene in *Culex pipiens* by microlitre plate tests. *Med Vet Entomol* 5:377–379.

Ferrari JA, Georghiou GP. 1981. Effects of insecticidal selection and treatment on reproductive potential of resistant, susceptible, and heterozygous strains of the southern house mosquito. *J Econ Entomol* 74:323–327.

Georghiou GP, Lagunes-Tejeda A. 1991. *The occurrence of resistance to pesticides in arthropods.* Rome, Italy: Food & Agriculture Organization of the United Nations.

Georghiou GP, Malik JI, Wirth M, Sainato K. 1992. Characterization of resistance of *Culex quinquefasciatus* to the insecticidal toxins of *Bacillus sphaericus* (strain). *Univ Calif Mosq Control Res Annu Rep* 1992:34–35.

Georghiou GP, Taylor CE. 1977. Genetic and biological influences in evolution of insecticide resistance. *J Econ Entomol* 70:319–323.

Lacey LA, Day L, Heitzman CM. 1987. Long-term effects of *Bacillus sphaericus* on *Culex quinquefasciatus. J Invertebr Pathol* 49:116–123.

Nielsen-LeRoux C, Charles J-F. 1992. Binding of *Bacillus sphaericus* binary toxin to a specific receptor on midgut brush-border membranes from mosquito larvae. *Eur J Biochem* 210:585–590.

Nielsen-LeRoux C, Pasquier CF, Charles J-F, Sinègre G, Gaven B, Pasteur N. 1997. Resistance to *Bacillus sphaericus* involves different mechanisms in *Culex pipiens* larvae. *J Med Entomol* 34:321–327.

Pei G, Oliveira CMF, Yuan Z, Nielsen-LeRoux C, Silva-Filha M-H, Yan J, Regis L. 2002. A strain of *Bacillus sphaericus* causes a slower development of resistance in *Culex quinquefasciatus. Appl Environ Microbiol* 68:3003–3009.

Rao DR, Mani TR, Rajendran R, Joseph AS, Gajanana A, Reuben R. 1995. Development of a high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. *J Am Mosq Control Assoc* 11:1–5.

Rodcharoen J, Mulla MS. 1994. Resistance development in *Culex quinquefasciatus* (Diptera: Culicidae) to *Bacillus sphaericus. J Med Entomol* 87:1133–1140.

Rodcharoen J, Mulla MS. 1997. Biological fitness of *Culex quinquefasciatus* (Diptera: Culicidae) susceptible and resistant to *Bacillus sphaericus. J Med Entomol* 34:5–10.

Roush RT, Hoy MA. 1981. Laboratory glasshouse and field studies of artificially selected carbaryl resistance

in *Metaseiulus occidentalis*. *J Econ Entomol* 74:142–147.

Rowland M. 1991. Behavior and fitness of γHCH/dieldrin resistant and susceptible female *Anopheles gambiae* and *An. stephensi* mosquitoes in the absence of insecticide. *Med Vet Entomol* 5:193–206.

Silva-Filha MH, Nielsen-LeRoux C, Charles J-F. 1997. Binding kinetics of *Bacillus sphaericus* binary toxin to midgut brush border membranes of *Anopheles* and *Culex* spp. mosquito larvae. *Eur J Biochem* 247:754–761.

Silva-Filha MH, Regis L. 1997. Reversal of low-level resistance to *Bacillus sphaericus* in a field population of the southern house mosquito (Diptera: Culicidae) from an urban area of Recife, Brazil. *J Econ Entomol* 90:299–303.

Silva-Filha MH, Regis L, Nielsen-LeRoux C, Charles J-F. 1995. Low-level resistance to *Bacillus sphaericus* in a field-treated population of *Culex quinquefasciatus* (Diptera: Culicidae). *J Econ Entomol* 88:525–530.

Sinègre G, Babinot M, Quermel J-M, Gaven B. 1994. First field occurrence of *Culex pipiens* resistance to *Bacillus sphaericus* in southern France. Proceedings of the 8th European Meeting of the Society for Vector Ecology. 1994 September 5–8; Barcelona, Spain. Santa Ana, CA: Society for Vector Control. p 17.

Suleman M, Reisen WK. 1979. *Culex quinquefasciatus* Say: life table characteristics of adults reared from wild-caught pupae from north west frontier province. Pakistan. *Mosq News* 39:756–762.

Thomas JG, Brazzel JR 1961. A comparative study of certain biological phenomena of a resistant and susceptible strain of the boll weevil, *Anthonomus grandis*. *J Econ Entomol* 54:417–420.

Wirth MC, Georghiou GP, Malik JI, Abro GH. 2000. Laboratory selection for resistance to *Bacillus sphaericus* in *Culex quinquefasciatus* (Diptera: Culicidae) from California, USA. *J Med Entomol* 37:534–540.

Yuan ZM, Zhang YM, Liu EY. 2000. High-level field resistance to *Bacillus sphaericus* C3-41 in *Culex quinquefasciatus* from southern China. *Biocontrol Sci Technol* 10:43–51.