Gezira, Sudan was cross-resistant to pyrethroids (Davidson and Curtis 1979). A DDT-selected *An. stephensi* strain from Kasur, Pakistan, when further selected with DDT plus chlorophenyl plus piperonyl butoxide, showed pyrethroid resistance (Omer et al. 1980).

Field trials carried out with pyrethroid compounds for control of house flies showed an increase of resistance to pyrethroids (Keiding 1980, Sawicki et al. 1981, MacDonald et al. 1983), but a similar trial carried out with permethrin in 9 dairies in California and New York did not provide evidence of resistance. But when such a population was subjected to high selection pressure of permethrin, not only resistance to permethrin developed rapidly, but the resistance level to organophosphorus compounds and DDT also increased (Scott and Georghiou 1985). Immigration of susceptible house flies from the neighboring areas and some other factors such as existence of refuge could have been responsible for not precipitating resistance in the field during 3 years. The finding that multiresistant *An. culicifacies* populations remained highly susceptible to deltamethrin is an interesting phenomenon. The monitoring of the susceptibility levels shown by multiresistant *An. culicifacies* populations to deltamethrin should be carried out as this compound is used against cotton pests in India and in such areas *An. culicifacies* is the main vector of malaria.

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TOXICITY IN CARCASSES OF *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS*-KILLED *AEDES AEGYPTI* LARVAE AGAINST SCAVENGING LARVAE: IMPLICATIONS TO BIOASSAY

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Serotype H-14 of *Bacillus thuringiensis* (var. *israelensis*, or *B.t.i.*) was discovered a decade ago (Goldberg and Margalit 1977). Its mosquito larvicidal activity has been extensively studied since then and exploited as a specific and efficient biological control agent (Arata et al. 1978, Margalit et al. 1983, Kirschbaum 1985). The δ-endotoxin responsible for this activity is produced during sporulation of this gram-positive bacterium and accumulated as a parasporal, amorphous crystal in the sporangium (Bulla et al. 1980). The high specificity of the toxin and the absence of variants developing resistance to it led to optimism with regard to control of vectors of lethal diseases. This enthusiasm faded somewhat with the recognition that the toxic activity has low persistence in natural ponds (e.g., Margalit et al. 1983).

Recently, Larget-Thiery (1984) demonstrated that successive additions of *Culex pipiens* (Linn.) larvae to a jar, initially inoculated with *B.t.i.* spores, preserved toxicity and a high concentration of *B.t.i.*-colony formers in the jar for 60 days, provided the dead larvae were not removed. Since ingested *B.t.i.* spores are known to germinate, to multiply and to

sporulate in the carcass of the *Aedes aegypti* (Linn.) larva they killed (Aly et al. 1985, Ohana[1]), our working hypothesis was that the toxic activity persisted due to cannibalistic behavior of the larvae (McIver and Siemicki 1977).

Aquatic dipteran larvae usually feed by filtering the water in which they dwell (Dadd 1971, Hopkins and Ramoska 1981). Mosquito larvae are known to ingest particles within the size range of 1–100μm (Wallace and Merritt 1980). In addition to their filter-feeding behavior, we demonstrate here that *Ae. aegypti* larvae can efficiently obtain nourishment from carcasses of their own and of related species. Carcasses of larvae killed by *B.t.i.* are shown to become toxic themselves to ingesting larvae.

In preliminary experiments, one or more carcass(es) of *Ae. aegypti* larva(e), killed by *B.t.i.* about 24 hr earlier, were rinsed in sterile water and transferred to a beaker containing several new larvae. The act of cannibalism ended within minutes, when severed heads were all that remained of the carcasses (Fig. 1D). The primary attack was usually at either the anal segment and its gills (Fig. 1A) or the thoracic segments (Fig. 1E) of the prey, and then proceeded systematically toward (Fig. 1A-D) or away from (Fig. 1E-G) the head, respectively. When a fresh carcass with a stiff exoskeleton was supplied, it seemed to have been sucked and drained and its cuticular shell discarded (not shown). Larvae which were actively gnawing or sucking on dead larvae, changed their

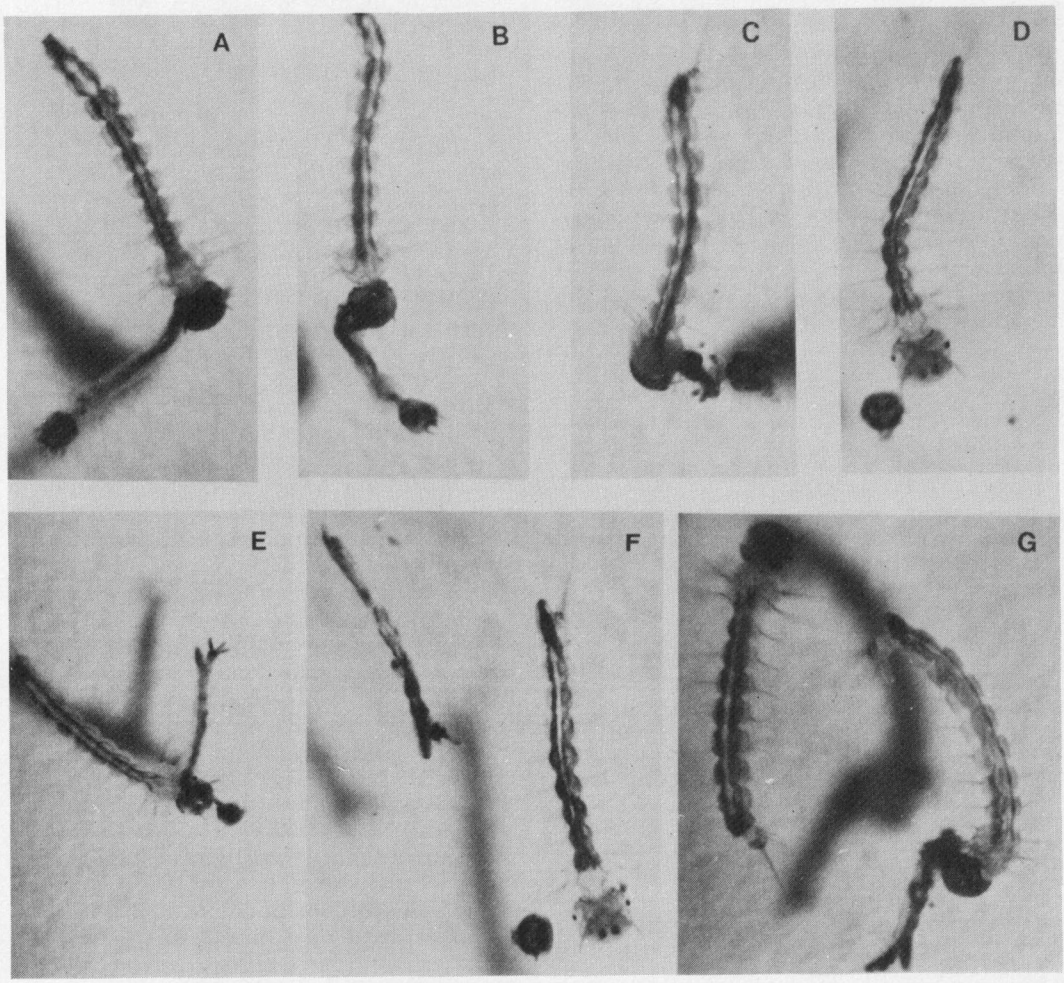

Fig. 1. Cannibalism of *Aedes aegypti* larvae. Third instar larvae were treated in water with sporulating *B.t.i.* powder at a concentration (4μg/ml) which killed them within an hour; the carcasses were incubated for 24 additional hrs at 25–30°C and then introduced to fresh larvae. Scavenging activity was photographed under a binocular microscope at a final magnification of between 8–12×.

normal mode of movement at about 30 min, and finally died (death defined as complete immobilization) about half an hour later. The transition period was distinguished by slowed movement with periods of trembling extending longer and longer as time passed on, and will be described in detail after a thorough investigation.

In order to rule out the possibilities that ingested *B.t.i.* excrete a larval chemoattractant or that any dead larva (*not* intoxicated) is poisonous, freshly killed larvae were introduced. Although they were ingested, no sign of trembling nor death was observed among the scavengers for at least 24 hr. In fact, *Ae. aegypti* is able to fully develop on carcasses as the only food source (Table 1). Fifteen newly-hatched larvae were rinsed in sterile water and transferred, each to a separate beaker containing 50 ml of either sterile water (10) or sterile food solution (5). Five beakers with water were supplemented, each with one carcass every two days (during the first week) or daily (afterwards). Developmental stages were recorded daily (in 24 hr intervals), during incubation at 25–30°C. The full development to adults was evident, albeit slower than on Pharmamedia. In unsupplemented water, the newly-hatched larvae did not develop further but rather died after several days, probably of starvation (Table 1).

A series of experiments were carried out to determine how long it takes for a larva intoxicated by scavenging a *B.t.i.*-killed larva to become toxic itself. Twenty-four hr old carcasses of *B.t.i.*-killed third instar *Ae. aegypti* larvae were rinsed with sterile water and introduced individually into beakers, each containing one third instar larva in 10 ml water. Each scavenger larva (dead or alive) was transferred at a given time after cannibalism to a beaker with another third instar larva (secondary scavenger), the behavior of which was continuously followed at 27 ± 2°C. The

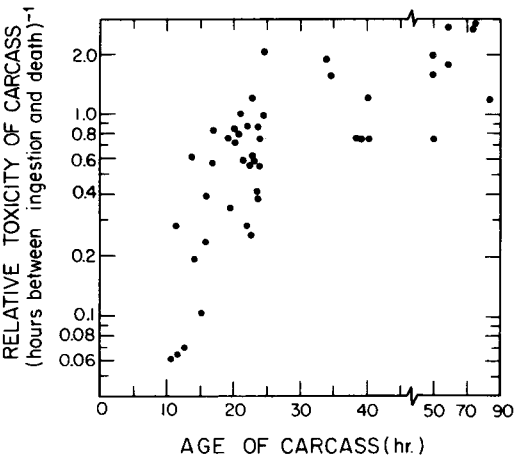

Fig. 2. Development of toxicity in intoxicated larvae. Reciprocal of time it took a secondary scavenger to die after ingesting a primary scavenger's carcass, as a function of carcass age.

relative toxicity of the primary scavenger's carcass, expressed as the reciprocal of time it took its scavenger (secondary) to die after ingestion, was plotted on a logarithmic scale as a function of its age (Fig. 2). Larvae which ingested the primary scavenger earlier than 10 hr did not die during at least 15 additional hours. The toxic activity of the carcass developed afterwards exponentially and reached a plateau at around 30 hr at room temperature (Fig. 2). Whether or not this rate of development is a function of the history of the original *B.t.i.*-killed larva, of temperature, of larval age etc., remains to be seen.

Our results show that the toxic activity does not pass (activated (Armstrong et al. 1985) or not) through the carcass to the scavenger as suggested (Larget-Thiery 1984), but rather develops in the carcass. The rate of development, though, was faster than expected, be-

Table 1. Development of *Aedes aegypti* larvae on carcasses as the only food source.

		Day of appearance of developmental stage														
		With carcasses					Pharmamedia*					Water				
Stage	Larva number	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Instar 1												D-2	D-4	D-3	D-3	D-1
Instar 2		2	2	2	2	2	2	D-2	2	2	2					
Instar 3		5	4	4	4	4	3		3	3	3					
Instar 4		8	8	8	8	8	4		4	4	4					
Pupa		10	D-9	10	10	10		5	5	5	5					
Adult		11		11	12	11	6		7	7	6					

D-i, death of the larva or pupa observed at the i-th day.

* Pharmamedia (Traders Protein, USA), 0.2–1.0 mg/ml in sterile aqueous solution. (This is the flour derived from cotton seeds after the linters, hull and oil have been removed.)

cause vegetative *B.t.i.* cells are non-toxic, while spores were observed inside the carcass microscopically after 30 hr but not after 24 hr following cannibalism of *B.t.i.*-killed larvae (data not shown). [Heat-resistant colony-forming *B.t.i.* were not found (Aly et al. 1985, Ohana[1]) in carcasses earlier than one day after larval death upon *B.t.i.* spore ingestion.] These observations raise several questions: Do larvae which escape intoxication by δ-endotoxin destroy ingested vegetative *B.t.i.*? Do conditions which prevail in *B.t.i.*-killed carcasses allow amplification or commitment shift of toxin production, or dissociation of sporulation from δ-endotoxin gene expression? There is an apparent paradox in combining these two questions; a resolution of which may open new avenues for raising persistence of toxicity, for gene expression research in *B.t.i.*, for investigating the mechanism of sporulation control and for studies of prey-predator/scavenger interactions in this biological system.

Preliminary observations indicate that carnivorous behavior is not specific to *Ae. aegypti*, but rather is more general in nature: larvae of *Culex pipiens* and of *Culiseta longiareolata* (Macquart) fed on and were intoxicated similarly by *B.t.i.*-killed *Ae. aegypti* larvae. Furthermore, carcasses of larvae of these organisms were similarly ingested by *Ae. aegypti* larvae and were toxic if killed by *B.t.i.* σ-endotoxin. Several dipteran species other than *Ae. aegypti* have been reported to feed carnivorously, in addition to filter-feeding (Chapman 1969), and blackfly larvae were reported (Wu 1931) to cannibalize. Other investigators appear to be aware of the cannibalistic tendencies of *Ae. aegypti* larvae (McIver and Siemicki 1977, Rishikesh and Quelennec 1983, G.B. Craig, Jr., personal communication), but the phenomenon has never been extensively described in the literature.

Standardized toxicity bioassays are performed in beakers with serial dilutions, each with 20 larvae, and percentage of survivors scored after 24 hr (Rishikesh and Quelennec 1983). This procedure may result in overestimating toxicity, in light of our observations (Fig. 2), as follows: some of the survivors at around LC_{50} (median lethal concentration) will probably feed upon the dead larvae. Cannibalism at 15 hr kills the scavenger 3–5 hr later, and at 20 hr, 1–2 hr later. Thus, the number of survivors at 24 hr is reduced, and the apparent toxicity is increased depending upon the cannibalistic activity of the sampled larvae participating in the bioassay. We therefore recommend that such bioassays be terminated not later than 18 hours.

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TOXORHYNCHITES RUTILUS SEPTENTRIONALIS FEEDING ON TREE SAP

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Consumed carbohydrates appear to be the major source of energy for mosquito flight and routine metabolic maintenance (Hocking 1953, Nayar and Van Handel 1971). These carbohydrates are obtained from a variety of sources including floral and extra-floral nectaries, honeydew, and fruit juices (Haeger 1955, Downes 1958, Sandholm and Price 1962, Bidlingmayer and Hem 1973, Grimstad and DeFoliart 1974). This report describes the use of tree sap as a source of plant juices by the mosquito *Toxorhynchites rutilus septentrionalis* (Dyar and Knab).

On May 17, 1986, an 18 cm diameter black oak (*Quercus velutina*) with a fresh cut in the bark was observed in a large mixed hardwood forest located in Chicot State Park (Evangeline Parish) Louisiana. Several trees with buttress roots or other water-holding cavities were located in the general area. The cut in the bark of the oak tree was ca. 8 cm long × 1.5 cm deep, and penetrated into the vascular tissue. Large quantities of liquid sap were leaking from the cut and dripping down the bark of the tree.

Honeybees, a variety of butterflies and sphingid moths, beetles (Tenebrionidae, Staphylinidae, Erotylidae), ants, and flies (Drosophilidae, Calliphoridae, Muscidae) were actively feeding on the leaking sap, which had a strong acetic acid aroma and appeared to be fermenting. At 1730 hr, a male *Tx. rutilus septentrionalis* landed on the tree and walked toward the cut while probing the bark with the proboscis. When it reached the cut containing the liquid sap, it probed into the fluid, then remained in a position with the proboscis in the fluid for ca. 10 min. It then walked 5 cm from the cut and remained motionless for ca. 30 min, until disturbed by the author.

This observation indicates that mosquitoes may feed upon tree sap that is liberated by some type of trauma. Also, it suggests that the mosquito located the sap using olfactory cues, since no visual cues indicative of a nectar source could have led the mosquito to that site. Several volatile chemicals, including ethanol, methanol, and acetaldehyde, are produced by fermenting sap (Moeck 1970). If olfactory cues are used in the location of plant juices by mosquitoes, it may be possible to study nectar-feeding periodicities by a modification of the technique known as "sugaring," which consists of smearing a fermenting mixture of sweet liquids on the bark of a tree (Borrer et al. 1981).

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