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Seed pods of the carob tree *Ceratonia siliqua* are a favored sugar source for the mosquito *Aedes albopictus* in coastal Israel

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ABSTRACT

The sugar feeding of *Aedes albopictus* was studied. In outdoor cages they fed avidly on carob (*Ceratonia siliqua* L., Caesalpiniaceae) seed pods soiled with sugary exudates. Feeding was either from the surface or by piercing the tissue which is indicated by the presence of plant tissue residues in the gut. More than 90% were sugar positive and about a third had plant tissue residues in the gut after overnight exposure. Similar exposure to clean undamaged seed pods resulted in about two thirds sugar positive mosquitoes and all of these had plant-tissue residues in the gut.

In an irrigated, field site, with abundance of sugar sources in the summer 68% of the females and 75% of the males were sugar positive whereas, 1.2% of the females and no males had plant tissue in the gut. The proportion of sugar positive mosquitoes was similar in the autumn and plant tissue was not found in 150 females and 13 males. In the dry site in the summer 42% females and 33% males were sugar positive while 22% females and 33% males contained plant tissue. In the autumn 73% females and 80% males were sugar positive while 2% females and none of the males contained plant tissue.

1. Introduction

Aedes albopictus (Skuse, 1895), a recent invasive species in the Mediterranean (Adhami and Reiter, 1998), was observed in Israel for the first time during 2002 at the southern coastal plain (Pener et al., 2003) from where it spread in the north to the border of Lebanon and in the south to Gaza. In the western, Mediterranean parts of the central mountain ridge it is presently found from the foothills up to Jerusalem, 800 m above sea level (unpublished data of the authors). Although *Ae. albopictus* is well studied with numerous publications on its ecology, vector capability, and blood feeding behavior (review by Gratz, 2004), the sugar feeding behavior of this species has been largely ignored. *Ae. albopictus* is known to readily feed on sugar in the laboratory, yet little is known about its sugar feeding habits in nature, and its ecological significance (Xue et al., 2008).

Sugars originating from plants, especially floral nectars, are a staple diet of mosquitoes (Hocking, 1968; Downes, 1958; O'Meara, 1987; Yuval, 1992). Mosquitoes also obtain sugars from extra floral nectaries on stems and at the base of leaves (Haeger, 1955; Nielsen and Greve, 1950), exudates from damaged plants (McCrae et al., 1976; Mogy and Miyagi, 1989; Nasci, 1986) and by directly piercing undamaged plant tissues (Abdel-Malek and Baldwin, 1961; Schlein and Müller, 1995; Müller and Schlein, 2005). There are few reports of mosquitoes feeding on honeydew (Patterson et al., 1969; Nielsen and Nielsen, 1958) and even less on fruit (Joseph, 1970). In this study we describe, apparently for the first time, observations of mosquitoes feeding on seed pods and their exudates. In the Mediterranean sugar sources like flowers and honeydew are rare during the summer months, while at the same time both wild and domestic fruit are common (Danin, 1988). The seed pods of the carob tree (*Ceratonia siliqua* L., Caesalpiniaceae) take a full year to develop with the main growth period from April to June followed by ripening and slow desiccation which changes the color of the seedpod from green to brown (Bosch et al., 1996). Nowadays, carob is also found in most areas in the Americas, Africa, Asia and Australia with suitable Mediterranean-like climate (Winer, 1980). Carob pods have traditionally been used as fodder for animals and for human consumption (Louca and Papas, 1973; Renfrew, 1973). Before sugar cane and sugar beets were widely available, carob provided ingredients for popular beverages and a wide variety of food products. It was also among the first horticultural crops used for the production of industrial alcohol by fermentation in several Mediterranean countries (Özcan et al., 2007; Zohary and Spiegel-Roy, 1975). Though the pulp is still used for numerous food byproducts the main interest is currently in the seeds that are used for gum extraction.

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The objective of this study was to investigate the sugar feeding habits of *Ae. albopictus*, particularly to substantiate our earlier observations of extensive feeding on carob seed pods. Our goal in this and other studies on sugar feeding behavior of mosquitoes is to identify attractive sugar sources that can be useful for mosquito control.

2. Material and methods

2.1. Field sites

Experiments and observations were carried out at two sites in the Mediterranean zone of Israel near Haifa. One site was a wasteland about 20 km south of Haifa on the coastal plain. The site is an open area about 40 m above sea level with some scattered olive, pistachio and carob trees. The undergrowth is continually grazed by goats and sheep. Three hundred meters to the west there is an irrigated banana plantation with an irrigation ditch in which *Ae. albopictus* was breeding as well as in irrigation puddles in the plantation. In summer, in June, there were no visible sugar sources like flowers, fruit and honeydew within a radius of 400 m around a group of carob trees that were bearing seed pods. In the autumn in October most carob seed pods were harvested and the remaining seed pods were dried up and often moldy. At the same period there were in the area many ripe wild growing honey-melons and grapes while the only flowering plant was the common perennial herb *Inula viscosa* L. Compositae (Asteraceae). Most of the leaves of *I. viscosa* were contaminated with honeydew excretion of the aphid *Aphis gossypii* Glover, Aphidae.

The second site includes irrigated gardens and parkland which are located at the outskirts of the Carmel Center, a residential neighborhood of Haifa 250 m above sea level. The site is on the slopes of Mount Carmel Ridge facing the Mediterranean Sea to the west. In the irrigated gardens and parkland in summer and autumn there were numerous flowering ornamental herbs, shrubs and trees and honeydew of several aphid species was common on the thick undergrowth. Common ripe fruits at the time were figs (*Ficus carica* L., Moraceae), pomegranates (*Punica granatum* L. Lythraceae), carob seed pods, loquat (*Eriobotyra japonica* Lindl. Rosaceae) and sabras (*Opuntia ficus-indica* L., Cactaceae). *Ae. albopictus* was breeding in irrigation puddles, ornamental ponds, neglected containers and some potted bromeliads. *Culex pipiens* L. and *Cx. perexiguus* Theobald were common at both sites except for *Ae. albopictus*. There were also a few *Aedes caspius* (Pallas), *Aedes detritus* (Haliday) and *Culiseta longiareolata* (Macquart) at both sites.

2.2. Observations in the field

In late spring 2009 we observed a group of carob trees heavily infested with the moth *Myelois ceratoniae* H. (Piralidae, Lepidoptera) near a known breeding habitat of *Ae. albopictus.* The maturing pods were damaged by the larvae, resulting in a massive excretion of a sweet, sticky aromatic, fermenting liquid which attracted numerous diurnal and nocturnal insects, including mosquitoes and their feeding schedule was observed. During daylight the mosquitoes were clearly visible while at darkness they were watched with infrared binoculars with 3× magnification (IR-Fernrohr Model 51 ZUB, AEG & Leica, Solms, Germany) and a Starlight Amplifier night vision goggles magnification 1:1 (Model 5157AA, ORTEC Electro Optics Industries, Rehovot, Israel). At the wasteland site the pods of a single carob tree were observed (26 and 27th of June) while in the garden and parkland site mosquitoes were looked for on the local flowers, fruit and honeydew-soiled vegetation (28 and 29th of June). Mosquitoes were monitored at both sites during two consecutive days in June from 1 h before sunrise (4:30 AM) until 3 h after sunset (23:00 PM), with observations of 10 min every hour. Samples for species identification of feeding mosquitoes were caught with an entomological net and a suction tube.

2.3. Collection of mosquitoes in the field

Mosquitoes in both sites were collected in the field by sweep net catches from 17:00 to 21:00 in the summer (2.7.2009 to 4.7.2009) and the autumn (5.10.2009 to 7.10.2009). Each day a random sample of 50 females was tested for sugar (cold anthrone test Van Handel, 1972) and a similar sample was examined for the presence of plant tissue in the gut (Schlein and Müller, 1995). A group of 150 females and the few collected males were processed in each test. Mosquitoes were either immediately treated or frozen at −70 °C.

2.4. Testing mosquitoes for sugar and plant tissue feeding

The sugar content in the gut of mosquitoes was evaluated by the cold anthrone test for fructose (Van Handel, 1967, 1972) as modified by Schlein and Jacobson (1994). The reaction solution contained 0.15% anthrone (Sigma, St. Louis, MO, U.S.A.) w/v in 71.7% sulphuric acid. Each mosquito was placed in the well of a flat-bottomed microtiter plate and wetted with 20 μl of 100% ethanol. Aliquots of 200 μl of reaction solution were added to the wells and the specimens were crushed with a glass rod that was repeatedly washed with water and wiped. After incubation for 60 min at 25 °C the crushed mosquitoes were removed from the wells under a dissecting microscope and sugar positive reactions were verified by color change. For identification of plant tissue feeding the slightly modified method of Schlein and Müller (1995) was employed. Fresh staining solution of 0.1% calcofluor (Fluorescent brightener 28, White M2R, CX.I. 40622, Sigma) in 0.45% saline, adjusted to pH 8 with NaOH, was prepared on a daily basis. Mosquito guts were dissected on microscopic slides in several drops of the staining solution, mounted on other microscopic slides in a drop of the staining solution and covered with cover slips. Prior to use, all the slides and cover glasses had been passed through the flame of a Bunsen burner to eliminate fluorescing particles of paper and cloth. Gut preparations were examined under a phase contrast-fluorescent microscope at a wave length of 360–440 nm, with a Wr 2B filter (Zetopan, Reichert, Vienna) to detect calcofluor-stained cell-wall cellulose particles (Kasten, 1980).

2.5. Experiments with caged mosquitoes

Ae. albopictus colony originating from specimens caught in the coastal plain of Israel was set up and maintained, from mid-June to mid-July 2009, under natural conditions in the garden and parkland site. The mosquitoes were maintained in shade, in 150 cm × 80 cm × 80 cm mosquito cages. The adults of the synchronized colony were given water and 10% sucrose solution ad libitum for four days after emerging and then they were starved with water only for the following two days. The same type of cages was later used for the feeding experiments. Several hundred mosquitoes of both sexes were used in three repetitions of each experiment to either four damaged carob seed pods soiled with fermenting juices (as found in the field) or to four undamaged carob seed pods which had been previously washed with distilled water. The carob seed pods were specifically collected from the observation site for each repetition. Mosquitoes were exposed to the pods and water from the afternoon (15:00) to the morning of the following day (9:00). After removing the water and the seed pods from the cages, the cages were placed in large heavy duty plastic sacks in which the mosquitoes were anesthetized with 10 ml of ethyl acetate soaked in a piece of cotton. Mosquitoes were examined immediately or