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ANALYSIS OF NECTAR AND HONEYDEW FEEDING IN *AEDES* AND *OCHLEROTATUS* MOSQUITOES

CURTIS B. RUSSELL AND FIONA F. HUNTER[1]

Department of Biological Sciences, Brock University, St. Catharines, Ontario L2S 3A1, Canada

ABSTRACT. Most research has investigated floral nectar as a source of carbohydrates for mosquitoes and has ignored homopteran honeydew. We have assessed the prevalence of honeydew and nectar feeding in 7 species of mosquitoes collected from Algonquin Provincial Park, Ontario, Canada. In total, 403 individuals were analyzed by thin-layer chromatography, with melezitose and stachyose as honeydew-indicator sugars. From the 403 individuals, 214 contained sugars, of which only 8.8% had the honeydew-indicating sugars. We conclude that female *Aedes* and *Ochlerotatus* mosquitoes in Algonquin Provincial Park seem to feed infrequently on honeydew.

KEY WORDS *Aedes, Ochlerotatus,* nectar, honeydew, thin-layer chromatography, light-traps, host-seeking

INTRODUCTION

Female mosquitoes are well known for their bloodfeeding habits, but in many species both sexes may consume sugar for flight energy and survival (Nayar and Sauerman 1975). Their main source of sugar generally is believed to be floral nectar, with extrafloral nectaries, honeydew, tree sap, rotting fruit, and sugar cane utilized only rarely (Foster 1995). Recently Burkett et al. (1999) showed that honeydew feeding may be important for some species but not for others. For example, in north-central Florida, 57% of *Anopheles quadrimaculatus* (Say) and 31% of *Culiseta melanura* (Coquillett) had fed on honeydew, whereas only 10% of *Coquillettidia perturbans* (Walker) and 7% of *Psorophora ferox* (von Humbolt) contained honeydew. Apart from the study by Burkett et al. (1999), little is known about the sugar preferences of mosquitoes and what effect honeydew vs. nectar might have on a mosquito's life.

In other bloodsucking Diptera such as Simuliidae and Tabanidae, homopteran honeydew has been found to be an important sugar source (Burgin and Hunter 1997a, 1997b; Janzen and Hunter 1998; Ossowski and Hunter 2000). The current study was designed to examine whether mosquitoes in the genera *Aedes* and *Ochlerotatus* use honeydew as a sugar source.

Honeydew is a sugary liquid excreted by homopterans that feed on plant phloem sap (Owen 1978, Kandler and Hopf 1980, MacVicker et al. 1990). This substance, called manna when solidified, contains an assortment of oligosaccharides, including the trisaccharide melezitose (Lombard et al. 1984, Henneberry et al. 1999).

Previous studies have shown that melezitose can be used as an indicator sugar for honeydew feeding by dipterans (MacVicker et al. 1990, Burgin and Hunter 1997a, Hunter and Ossowski 1999). The tetrasaccharide stachyose also has been used as a honeydew indicator sugar in previous studies conducted in Algonquin Provincial Park (Burgin and Hunter 1997a, Janzen and Hunter 1998, Hunter and Ossowski 1999). Thus, we used both melezitose and stachyose as honeydew-indicator sugars in the current thin-layer chromatography (TLC) study.

MATERIALS AND METHODS

Collection of adult mosquitoes: All collections took place in Algonquin Provincial Park, Ontario, Canada (45°34′N, 78°1′W) between June 12 and July 2, 2000. Two different collection techniques (light trapping and human bait) were used to maximize both the diversity of species and the number of individuals captured. Bat Lake and Tote Road were chosen as light-trap sites because they represented 2 different habitat types. Bat Lake was a boggy region, dominated by ericaceous shrubs, whereas Tote Road was a dry, hardwood forest. Host-seeking adults were collected from human bait at the Lake Sasajewun Dam and the Mew Lake Campground. The Lake Sasajewun Dam site was a mixture of coniferous and deciduous trees. The Mew Lake Campground site was situated close to a stand of tamarack trees (*Larix laricina*) that were known to have honeydew-producing homopterans (*Adelges* sp.) feeding from them (Burgin and Hunter 1997a).

Light-traps: Light traps were Centers for Disease Control miniature black light traps (Model 512, John W. Hock Co., LaSalle, CA). Traps were operated from 7:00 p.m. to 7:00 a.m. In the mornings, the collection container was returned to the field laboratory at the Wildlife Research Station and placed directly into a −20°C freezer to kill the trapped insects. Female mosquitoes were later identified to species, placed into individually labeled 1.5-ml Eppendorf tubules, and stored at −20°C until they were transported to Brock University (St. Catharines, Ontario, Canada) for TLC analyses.

Collections with human bait: Collections of host-seeking mosquitoes were made on the same

[1] To whom correspondence should be addressed.

day from 7:00 to 8:00 p.m. at the Lake Sasajewun Dam and from 8:00 to 9:00 p.m. at Mew Lake. If a mosquito started bloodfeeding before capture, it was discarded. As mosquitoes landed on the subject, they were caught individually in 1.5-ml Eppendorf tubules. Specimens were freeze-killed immediately upon return to the field laboratory, identified to species, and stored at −20°C until used in TLC analyses.

Thin-layer chromatography plate preparation: For each collection method, collection site, and collection date, up to 20 individual mosquitoes were selected at random from all mosquitoes in the sample. Each individual was then removed from its 1.5-ml Eppendorf tubule, placed under a dissecting microscope, and allowed to thaw. The mosquito's legs, wings, and head were removed with fine forceps. The remaining body was placed in a new 1.5-ml Eppendorf tubule with 10 μl of distilled water and triturated with a glass pestle until no whole body parts could be recognized (~40 sec).

Thin-layer chromatography glass plates (20 × 20 cm) were coated with a 0.5-mm thickness of Sigmacell® type 20 powdered cellulose (Sigma Chemical Co., St. Louis, MO). Two different standards were prepared for use on the TLC plates. Both standards were made by placing 0.1 g of each sugar into 10 ml of distilled water. Standard set 1 consisted of fructose, glucose, sucrose, maltose, maltotriose, and melebiose. Standard set 2 consisted of turanose, melezitose, and stachyose. On each TLC plate, 28 sites were available for the application of a test sample at 0.6-cm intervals along the origin of the plate (~2 cm from the bottom of the plate). No spots were applied to the outer 1.5-cm edges of the plate.

Wiretrol® disposable microcapillary tubes (Drummond Scientific Co., Broomall, PA) with wire plungers were used to apply the standards and the mosquito samples. All test samples applied to the plate were of a 1.5-μl volume. Standard set 1 was applied to positions 1 and 14, and standard set 2 was placed on positions 15 and 28. The contents of individual mosquitoes were applied to positions 3–12 and 17–26.

The solvent for these experiments consisted of 15 ml of formic acid, 25 ml of methyl ethyl ketone, 35 ml of tertiary butanol, and 25 ml of distilled water. A volume of 100 ml of this mixture was used in the developing chamber; a fresh mixture was used to develop each plate. Once a plate had been spotted with the standards and the mosquito samples, it was placed in the developing chamber along with the solvent. The TLC plate was left in the chamber until the solvent front had reached the upper 0.5 cm of the plate. The plate was then removed from the chamber and allowed to air dry at room temperature until the solvent had completely evaporated.

To observe the different sugars present on the TLC plate, a mixture of urea (3 g), water-saturated 1-butanol (90 ml), and phosphoric acid (25 ml) was used. Plates were sprayed until saturated (but not glistening) with a glass atomizer from a distance of approximately 25 cm. Plates were allowed to dry for 10 min and were then heated with a flameless heat gun until all spots had appeared.

All spots, the solvent front, and the origin were traced onto an overhead acetate sheet. The high retention factor (hRf) values were determined with the following calculation: hRf = (100 × migration distance from origin to middle of sugar spot/migration distance from origin to solvent front).

Mosquitoes containing melezitose or stachyose were scored as being recently honeydew-fed, whereas mosquitoes containing fructose, glucose, or sucrose without the presence of melezitose or stachyose were scored as nectar-fed. Individuals containing an unknown sugar were scored as either honeydew- or nectar-fed depending on the other sugars present.

RESULTS

Species enumeration

Females of 5 species of *Ochlerotatus* (*Oc. canadensis* (Theobald), *Oc. communis* (De Geer), *Oc. excrucians* (Walker), *Oc. provocans* (Walker), and *Oc. sticticus* (Meigen)), and 2 species of *Aedes* (*Ae. cinereus* Meigen and *Ae. vexans* (Meigen)) were captured. The 2 most abundant species captured were *Oc. canadensis* and *Oc. provocans.*

Up to 20 female mosquitoes from each collection date, method, and site were analyzed individually by means of TLC, for a total sample size of 403. In some cases, 20 individuals were not available for a particular date, collection method, or site.

hRf values

The urea reagent did not react with maltose, maltotriose, or melebiose. The standards for turanose and sucrose migrated to positions that were virtually identical on the plates. Thus, it was not possible to determine if mosquito sugars that had hRf values of 41.1 ± 5.0 were sucrose, turanose, or both. These were scored as Suc/Tur (Fig. 1). The mean hRf values from the mosquito samples were generally slightly lower than those for the sugar standards, although not statistically so (Fig. 1). An unknown sugar (?? in Fig. 1) with a mean hRf value of 15.6 ± 4.2 was observed in 40 of the sugar-fed mosquitoes. This hRf value did not overlap or correspond to any of the standard sugars that were used. The unknown sugar migrated to a distance between the trisaccharide raffinose and the tetrasaccharide stachyose; this sugar is suspected to be either a trisaccharide or a tetrasaccharide.

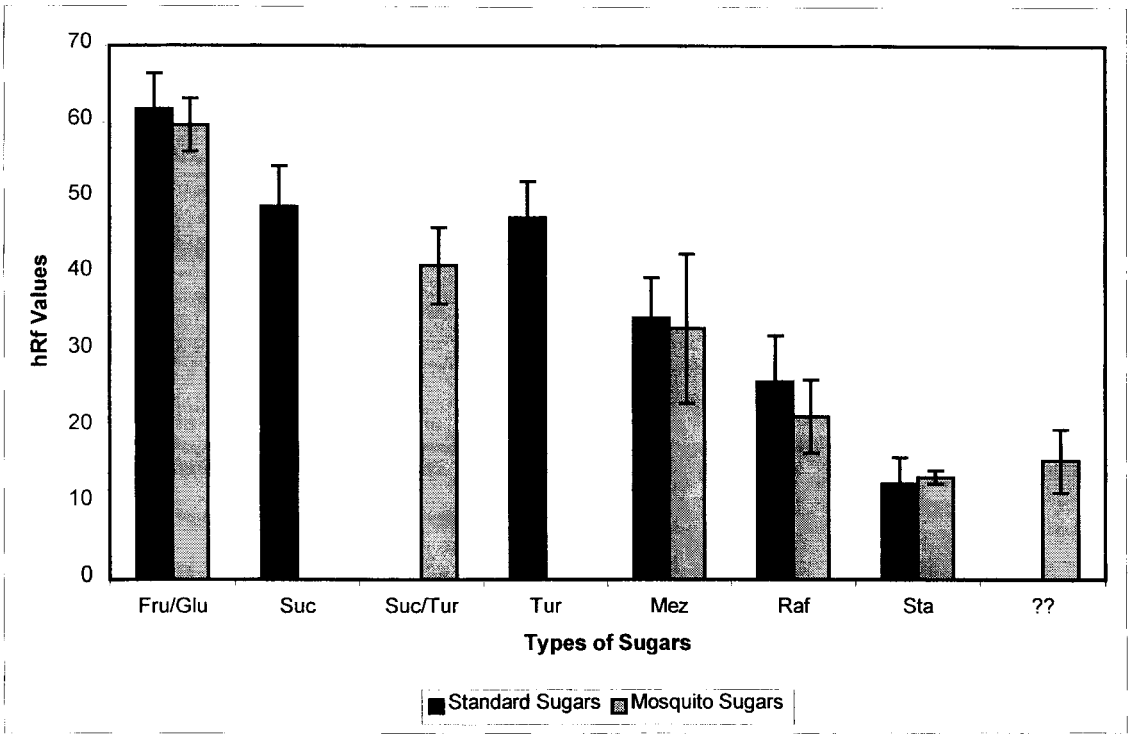

Fig. 1. Mean (±SD) high retention factor (hRf) values for the standard sugars (n = 48) and the sugars found in the individual mosquito samples (n = 214) collected in Algonquin Provincial Park, Ontario, Canada. Abbreviations for the sugars are: Fru, fructose; Glu, glucose; Suc, sucrose; Tur, turanose; Mez, melezitose; Raf, raffinose; Sta, stachyose; and ??, the unknown.

Sugar composition

Among the 403 female mosquitoes analyzed, only 214 (53.1%) contained sugars. Of these 214, only 19 (8.8%) tested positive for the honeydew-indicating sugars melezitose or stachyose. Thirteen different sugar combinations were observed among the individuals that tested positive for sugars (Table 1). The proportion of mosquitoes testing positive for sugars was similar among collection sites and methods: in the light traps, 56 (50.5%) of 111 at Tote Road vs. 41 (62.1%) of 66 at Bat Lake; from human bait, 55 (48.7%) of 113 at Mew Lake vs. 62 (54.9%) of 113 at Lake Sasajewun Dam.

Chi-square analysis

A contingency chi-square analysis was performed on *Oc. canadensis* and *Oc. provocans,* the 2 species with the most individuals, to determine if a difference existed in honeydew vs. nectar consumption between the 2 species. Data for all sites and both collection methods were pooled. Chi-square analysis indicated no significant difference between the 2 species in honeydew vs. nectar consumption (χ^2 = 0.20, df = 1, P = 0.6566). An additional chi-square analysis was conducted to determine whether a difference existed in honeydew vs. nectar consumption between mosquitoes captured host-seeking versus in light traps. Data for all species were pooled. This analysis indicated no significant difference between the 2 collection methods (χ^2 = 0.45, df = 1, P = 0.5028).

DISCUSSION

hRf values

The unknown sugar observed in this study had a mean hRf value of 15.6, which is very similar to an unknown sugar identified in Algonquin Park tabanids by Ossowski and Hunter (2000), which had an hRf value of 15.2. We tested the hypothesis that this unknown sugar was maltotriose by including it in standard set 1 but it was not detected by the urea reagent. The possibility exists that this unknown sugar could be another honeydew-indicating sugar. If true, this would increase the number of individuals that contained honeydew from 19 (8.8%) to 59 (27.6%).

Prevalence of honeydew vs. nectar feeding

Of the 214 mosquitoes that tested positive for sugar, only 19 (8.8%) contained the honeydew-indicating sugars melezitose or stachyose. We con-

Table 1. The 14 different sugar combinations found in the 7 different *Ochlerotatus* and *Aedes* species analyzed by thin-layer chromatography along with the number of individuals containing the honeydew, nectar, or no sugars.

Sugar composition[1]	No. individuals							
	Oc. canadensis	*Ae. cinereus*	*Oc. communis*	*Oc. excrucians*	*Oc. provocans*	*Oc. sticticus*	*Ae. vexans*	Total
No sugars	50	15	29	1	92	1	1	189
Fru/Glu	30	2	25	0	37	0	0	94
Suc/Tur	1	0	1	0	0	0	0	2
Fru/Glu, Mez	0	0	1	0	3	0	0	4
Fru/Glu, Raf	0	0	0	0	2	0	0	2
Fru/Glu, Raf, ??	0	0	1	0	0	0	0	1
Fru/Glu, ??	0	0	0	1	0	0	0	1
Fru/Glu, Suc/Tur, Mez	1	0	0	0	1	0	0	2
Fru/Glu, Suc/Tur, Raf	5	0	4	1	8	0	0	18
Fru/Glu, Suc/Tur, ??	0	0	1	0	2	0	0	3
Fru/Glu, Suc/Tur, Mez, Raf	2	0	1	0	2	0	0	5
Fru/Glu, Suc/Tur	13	0	6	0	19	0	1	39
Fru/Glu, Suc/Tur, Raf, ??	5	0	11	0	19	0	0	35
Fru/Glu, Suc/Tur, Raf, Sta	2	0	0	0	5	1	0	8
Total	109	17	80	3	190	2	2	403
Honeydew sugars	5	0	2	0	11	1	0	19
Nectar sugars	54	2	49	2	87	0	1	195
No sugars	50	15	29	1	92	1	1	189

[1] Fru, fructose; Glu, glucose; Suc, sucrose; Tur, turanose; Mez, melezitose; Raf, raffinose; ??, unknown; Sta, stachyose.

clude that the remaining mosquitoes (91.2%) had fed on floral or extrafloral nectar (Table 1). These results show that honeydew feeding is not very prevalent among *Ochlerotatus* and *Aedes* mosquitoes in Algonquin Park. This is an unexpected outcome, because it differs from the previous studies that have shown other biting flies (Simuliidae and Tabanidae) in Algonquin Park to have a much larger proportion of individuals feeding on honeydew. For example, Burgin and Hunter (1997a) found that 49.7% of *Simulium venustum* collected from an adelgid-infested tamarack stand had fed on honeydew and overall, about one third of black flies contained honeydew (Burgin and Hunter 1997c). Janzen and Hunter (1998) examined deer flies and found that 73.3% had fed on honeydew. Hunter and Ossowski (1999) analyzed horse flies and found that 38.9% at an abandoned airfield and 51.4% at a bog contained honeydew.

Ochlerotatus in the current study have honeydew feeding levels (~9%) similar to the results of Burkett et al. for *Coquillettidia* (10%) and *Psorophora* (7%), but different from their results for *Anopheles* (57%) and *Culiseta* (31%). We examined several different *Ochlerotatus* and *Aedes* species and found no evidence of species-characteristic honeydew feeding patterns. Concluding that members of the genus *Ochlerotatus* (along with genera such as *Coquillettidia* and *Psorophora*) may simply prefer nectar to honeydew is tempting. However, collections of mosquitoes from other habitats, geographic locations, and weather conditions are required before definitive conclusions are drawn. We currently are examining the effects of honeydew vs. nectar on life history traits such as longevity and fecundity. Further preliminary studies in our laboratory suggest that sugar meal source also has important implications for understanding flight performance (speed, distance, and duration) and vectorial capacity in biting flies.

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