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ABSTRACT Adult stable flies (*Stomoxys calcitrans* L.) are known to feed readily on sugars in the laboratory. However, little is known concerning the extent of stable fly sugar feeding in wild populations. We examined the frequency of sugar feeding in stable flies collected on Alsynite sticky traps in rural and urban environments. In addition, stable flies were visually examined to determine whether blood was present in the gut. In laboratory studies, sugars were detectable with the anthrone technique in stable flies for ≈3 d after being imbibed, and blood could be visually detected in the gut for 24–48 h after feeding. Twelve percent of the field-collected flies had detectable sugar with a higher percentage of the urban flies having sugar fed than the rural flies, 21 and 8%, respectively. Female flies sugar fed at a slightly higher rate than males, 13 versus 11%, respectively. Less than 1% of the field-collected flies had blood in their guts. The frequency of observable blood was slightly higher in flies collected in an urban environment compared with those collected in a rural environment and did not differ between male and female flies. The number of flies with both blood and sugar was slightly higher than would be expected based on the frequencies of each alone. Seasonal patterns of both sugar feeding and blood feeding were similar in the rural and urban environments; both peaked in the early summer, May to mid-June, and dropped through the summer and fall. Sugar feeding in the urban environment increased again in October.

KEY WORDS *Stomoxys*, adult feeding, sugar, blood

Stable flies, *Stomoxys calcitrans* L., are important pests of livestock. Damages to livestock production in the United States caused by stable flies were estimated to be $432 million in 1991 (Kunz et al. 1991). During the past 20 yr, stable flies have emerged as a pest of pasture cattle and confined animals (Hall et al. 1982, Campbell et al. 2001, Broce et al. 2005). Based on extrapolations from several sources, Taylor and Berkebile (2006) estimated losses in the United States to be closer to $1 billion per year.

Both male and female stable flies require blood feeding before successful mating (Anderson 1978), and females require blood for ovarian development (Kuzina 1942). Unlike nematocerous Diptera, which produce one egg batch per full blood meal (Sutcliffe et al. 1993), stable flies require three to five blood meals to complete the first ovarian cycle (Kuzina 1942, Chia et al. 1982) and three additional blood meals for completion of subsequent ovarian cycles (Chia et al. 1982). In addition to being needed for reproduction, blood feeding prolongs the life of stable flies (Moobola and Cupp 1978).

Stable flies will readily feed on sugars when offered in the laboratory and have been observed probing flowers in the field (Tseng et al. 1983). Jones et al. (1985) found between 3 and 23% stable flies collected at Florida beaches and dairies had fed on sugars, with twice as many females testing positive for sugars as males. Caged stable flies provided sugar ad libitum had lower reproductive success than those provided sugar only once or no sugar (Jones et al. 1992). Sugars do not support ovarian development or stimulate mating. However, stable flies provided sugar in the absence of blood can survive up to five times longer than those provided with water alone (Jones et al. 1992) but not as long as those provided blood (Moobola and Cupp 1978). When blood is provided ad libitum, sugar does not seem to increase fecundity or survival of stable flies (Jones et al. 1992). Other than observations of stable flies on flowers and the brief study of Jones et al. (1985) in Florida, little is known of the frequency of sugar feeding by wild stable flies.

If sugar feeding is an important component of stable fly biology, it could open several new avenues for research into stable fly biology and control. The purpose of this study was to evaluate the frequency of sugar feeding in wild stable flies collected on Alsynite sticky traps. Because stable fly feeding behavior may differ between rural environments where livestock provide an abundance of hosts for blood feeding and the urban environment where gardens and ornamental plantings may provide a greater abundance of nectar sources, flies were sampled from both environments.

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Materials and Methods

Laboratory Study. Stable fly larvae were reared in a media consisting of wheat bran (500 g), fish meal (115 g), wood chips (200 g), and water (1.6 liters). Immatures were allowed to develop for 12–14 d before the pupae were harvested. Four cages (30 by 30 by 30 cm) were prepared, each with 15 g of stable fly pupae (≈1,400 pupae). All cages were provided with water ad libitum and maintained at 24 ± 2°C. Two days after the initiation of adult emergence, remaining pupae were removed from the cages. Twenty-four hours after the pupae were removed, feminine pads (Stayfree nonscented feminine napkins, 23 by 7 cm; McNeil-PPC, Morris Plains, NJ) soaked in water, 10% sucrose solution, 10% corn syrup (CS, Always Save Light Corn Syrup; Associated Wholesale Grocers, Kansas City, KS; 80% carbohydrates, 30% sugars) solution or citrated beef blood (3.7 g sodium citrate/liter blood) were placed in dishes inside the cages. Flies were permitted to feed for 24 h, after which the pads were removed, leaving the cages with only water. Care was taken to avoid leaving any residue of blood or sugar on which the flies could feed after the dishes containing the pads were removed. Treatments were (1) water (control), (2) sucrose, (3) CS, (4) beef blood, (5) sucrose and blood, and (6) CS and blood. For the combination treatments 5 and 6, two pads were placed in each cage, one with blood and the other with the appropriate sugar. Approximately 50 flies were removed from each cage at the time the experimental diets were removed (time 0) and every 24 h until no live flies were left. Flies were kept in a −20°C freezer until analyzed. For analysis, flies were sexed and placed in 48-well plates (Greiner Bio One, Longwood, FL). Mid- and hind-guts were removed from those flies with visible blood meals and placed in lysis buffer for a future study on bloodmeal identification.

Anthrone Assay. The anthrone assay to detect sugar in stable flies was that of Van Handel (1972, 1985) with slight modifications. Flies were crushed in the 48-well plates, and 150 μl of anthrone reagent (76 ml concentrated sulfuric acid, 30 ml distilled water, 150 mg anthrone; Sigma-Aldrich, St. Louis, MO) was added to each well. Plates were maintained at room temperature (22°C) and observed at 30 min, 2 h, and 3 h. The presence of sugar was indicated by the anthrone reagent changing color from yellow to blue. For the laboratory test, intensity of anthrone reactions was determined by scoring the length of time required for the color change to become apparent. Flies were scored as 3 if blue appeared in <30 min, 2 if <2 h, 1 if <3 h, and 0 if no color change was observed at 3 h. In the field study, flies were scored as 1 or 0 depending on the appearance of a color change at 3 h. Plates were scored visually with a white background by the same individual to maintain consistency.

Stable Fly Field Collections. Stable flies were collected from 12 Broce Alsynite traps (Broce 1988). Six traps were located within the city limits of Lincoln, NE, referred to as the urban traps, and six traps were located at the University of Nebraska Agriculture Research and Development Center (ARDC) located near Ithaca, NE, referred to as the rural traps. Urban traps 1–3 were located at the urban–rural interface. Trap 1 was located in a residential backyard directly south of an interstate highway. Cropland, pasture, and small confined animal properties were present north of the highway. Trap 2 was located on a golf course near the maintenance building where mowers were frequently washed, resulting in accumulations of decomposing vegetation and moisture. Trap 3 was located in an industrial park near a small water impoundment. Trap 4 was located at the Folsum Children's Zoo adjacent to the pony rides. Traps 5 and 6 were in residential neighborhoods.

ARDC encompasses ≈4,000 ha of crop land and pasture. A 150 cow dairy and 1,300 head capacity research feedlot are located on the property, and a second feedlot, the Mead Cattle Company (MCC) with a capacity of 39,000 head, is located adjacent to the northern border. Rural traps 7 and 8 were located within 500 m of beef cattle feedlots. Trap 7 was 100 m north of the research feedlot, whereas trap 8 was located 500 m south of the larger MCC feedlot. Traps 9 and 10 were located in cropland, whereas traps 11 and 12 were located in pasture land.

Stable flies were collected for two consecutive 24-h periods per week from 9 May to 1 November 2006. Flies were kept in a −20°C freezer until dissected. A maximum of 24 flies per trap per 24-h collection was randomly selected for analysis. Flies were assayed for blood and sugar as indicated in the laboratory study.

Statistical Analysis. χ^2 tests for homogeneity (Proc Freq; SAS Institute 2004) were used to analyze the blood and sugar data relative to stable fly sex, trap locations, and habitat zones. A probability level of 0.05 was used for all tests.

Results

Laboratory Study. Control flies, those fed only water, did not survive ≥24 h. None of the flies provided water alone tested positive for sugar with the anthrone test nor was blood visible in any of their guts (Table 1). Flies fed sucrose produced a more intense anthrone reagent reaction than did those fed CS. The percentage of flies with detectable sugar was similar between 0 and 24 h after the sugar was removed. For sucrose, most of the flies still had detectable sugar after 48 h, and one third tested positive after 72 h. For CS, about one third of the flies tested positive at 48 h, and only a very small percentage tested positive after 72 h. Blood was visible in the digestive system of flies 24 h after the source was removed but not at 48 h for all three groups provided blood. None of the flies fed blood only tested positive for sugar, and the presence of blood did not seem to reduce the ability to detect sugar in flies fed both.

Field Study. A total of 38,417 stable flies were collected during the study, of which 7,206 were analyzed (Table 2). Sugar was detected in 12% of the stable flies. More flies collected in the urban environment had detectable sugar than those collected in the rural en-

Table 1. Numbers of stable flies with visible blood in the gut and/or sugar, 0–4 d after feeding in laboratory experiments

Treatment	Hours	Analyzed (*N*)	Blood fed [*N* (%)]	Sugar fed [*N* (%)]	Intensity mean ± SD
Control	0	48	0 (0.0)	0 (0.0)	0.00 ± 0.00
	24	0			
	48	0			
	72	0			
	96	0			
10% Sucrose	0	48	0 (0.0)	48 (100.0)	2.92 ± 0.40
	24	48	0 (0.0)	46 (95.8)	2.87 ± 0.61
	48	48	0 (0.0)	38 (79.2)	2.29 ± 1.24
	72	48	0 (0.0)	19 (39.6)	1.06 ± 1.37
	96	48	0 (0.0)	5 (10.4)	0.23 ± 0.72
10% CS	0	48	0 (0.0)	40 (83.3)	1.81 ± 1.10
	24	48	0 (0.0)	40 (83.3)	1.77 ± 0.99
	48	48	0 (0.0)	14 (29.2)	0.60 ± 1.09
	72	48	0 (0.0)	2 (4.2)	0.04 ± 0.20
	96	48	0 (0.0)	1 (2.1)	0.06 ± 0.43
Blood	0	48	48 (100)	0 (0.0)	0.00 ± 0.00
	24	48	27 (56.2)	0 (0.0)	0.00 ± 0.00
	48	48	0 (0.0)	0 (0.0)	0.00 ± 0.00
	72	48	0 (0.0)	0 (0.0)	0.00 ± 0.00
	96	48	0 (0.0)	0 (0.0)	0.00 ± 0.00
Blood and sucrose	0	48	48 (100.0)	46 (95.8)	2.79 ± 0.65
	24	48	13 (27.1)	46 (95.8)	2.83 ± 0.63
	48	48	0 (0.0)	37 (77.1)	2.02 ± 1.26
	72	48	0 (0.0)	19 (39.6)	1.06 ± 1.37
	96	48	0 (0.0)	4 (8.3)	0.19 ± 0.67
Blood and CS	0	48	47 (97.9)	20 (41.7)	0.63 ± 0.87
	24	48	15 (31.2)	23 (47.9)	0.77 ± 0.97
	48	48	0 (0.0)	4 (8.3)	0.19 ± 0.67
	72	48	0 (0.0)	2 (4.2)	0.04 ± 0.20
	96	48	0 (0.0)	0 (0.0)	0.00 ± 0.00

Intensity was based on the length of time needed for the anthrone reagent to begin changing color. Flies were scored as 3 for <30 min, 2 for <2 h, 1 for <3 h, or 0 for no change after 3 h.

vironment, 21 and 8%, respectively ($\chi^2 = 245.07$, df = 1, $P < 0.0001$). In the rural environment, flies collected in pastures had the highest frequency of sugar, followed by cropland and adjacent to feedlots, 10, 8, and 7%, respectively ($\chi^2 = 10.67$, df = 2, $P = 0.005$). The frequency of detectable sugar did not differ among the interface, residential, and zoo sites in the urban environment ($\chi^2 = 2.78$, df = 2, $P = 0.25$). Slightly more females had detectable sugar than males, 13 and 11%, respectively ($\chi^2 = 5.73$, df = 1, $P = 0.02$). The seasonal pattern of stable flies with detectable sugars was similar between the rural and urban environments, with the highest levels of detectable sugars being in the spring and dropping throughout the summer (Fig. 1B). Sugar feeding increased in the urban environment in the fall but remained low in the rural environment.

Blood was visible in 0.8% of the flies, and the frequency of flies with blood was higher in the urban environment ($\chi^2 = 4.0$, df = 1, $P = 0.04$). Comparison of the frequencies of flies with visible blood collected on rural traps located adjacent to feedlots, in cropland, and in pastures indicated no differences between those three agroecotypes ($\chi^2 = 0.97$, df = 2, $P = 0.62$).

Table 2. Numbers of field-collected stable flies with visible for blood in the gut and/or positive for sugar

	Trap	Environment	Collected (*N*)	Analyzed (*N*)	Blood fed [*N* (%)]	Sugar fed [*N* (%)]	Sugar and blood fed [*N* (%)]
Urban	1	Interface	317	231	2 (0.9)	64 (27.7)	1 (0.4)
	2	Interface	784	472	9 (1.9)	62 (12.9)	0 (0.0)
	3	Interface	826	371	2 (0.05)	119 (31.9)	0 (0.0)
	4	Zoo	5,356	1,069	11 (1.0)	214 (19.8)	9 (0.8)
	5	Residential	1	1	0 (0.0)	1 (100.0)	0 (0.0)
	6	Residential	23	23	1 (0.4)	3 (13.0)	0 (0.0)
	Total		7,309	2,189	25 (1.1)	463 (21.1)	10 (0.5)
Rural	7	Feedlot	6,840	946	4 (0.4)	60 (6.3)	1 (0.1)
	8	Feedlot	16,176	1,199	8 (0.7)	86 (7.2)	1 (0.1)
	9	Cropland	3,410	889	7 (0.8)	85 (9.6)	3 (0.3)
	10	Cropland	2,065	801	5 (0.1)	57 (7.1)	1 (0.1)
	11	Pasture	1,301	549	2 (0.4)	46 (8.4)	0 (0.0)
	12	Pasture	1,316	633	8 (1.3)	72 (11.4)	1 (0.2)
	Total		31,108	5,017	34 (0.7)	406 (8.1)	7 (0.1)
Grand	Total		38417	7,206	59 (0.8)	869 (12.1)	17 (0.2)

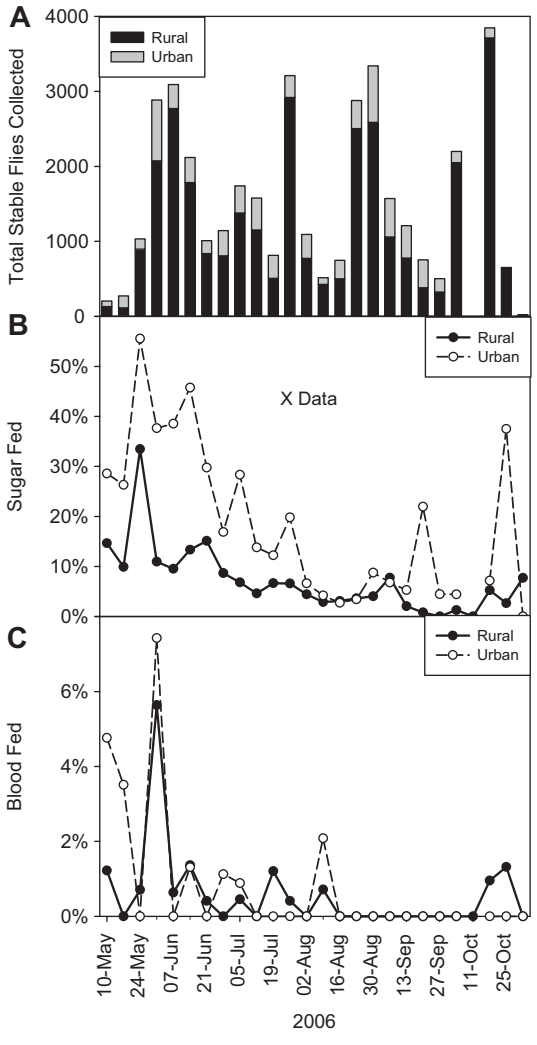

Fig. 1. Seasonal variation in stable fly collections and sugar and blood feeding in stable flies. (A) Total number of stable flies collected on 12 Alsynite sticky traps (6 rural and 6 urban) during two consecutive 24-h periods each week. Up to 24 flies per trap day were analyzed for the presence of sugar and blood. (B) Percentage of stable flies analyzed testing positive for sugars with the anthrone reagent. (C) Percentage of stable flies analyzed with visible blood in the gut.

In the urban environment, the number of flies with detectable blood did not differ between the zoo, residential, and urban–rural interface locations (χ^2 = 2.12, df = 2, P = 0.35). Overall, the number of male and female stable flies with visible blood did not differ (χ^2 = 0.46, df = 1, P = 0.50). The seasonal pattern of the percentages of flies with visible blood was similar in the rural and urban environments (Fig. 1C). The frequency of stable flies with blood was higher in the early season and decreased through the summer, with a small rise in the fall. The percentage of flies with both blood and sugar, 0.24%, was higher than that expected based on the frequencies of blood and sugar individually, 0.10% (χ^2 = 15.7, df = 1, $P < 0.0001$).

Discussion

In our laboratory study, we were able to detect sugar in most of the flies 24–48 h after feeding on sucrose and in a small proportion of the flies up to 96 h after feeding. This result differs from that observed by Tseng et al. (1983), who were able to detect sugar in flies for only up to 22 h and found sugar in only 50% of the flies 18 h after sugar feeding. The reason for this discrepancy is unclear because Tseng et al. (1983) used a range of concentrations of sucrose (10–50%) compared with our 10% sucrose. The anthrone reaction was more intense with flies fed sucrose than with those fed CS (Table 1). This was expected because the concentration of sugar in the 10% CS was 3%, less than one third as much as the 10% sucrose solution. Plant nectars contain from 8 to 40% sugars (Wykes 1953). The percent of flies with detectable sugars did not differ between the 0- and 24-h postfeeding samples with either sugar solution. Blood was visible in the gut of the flies for only ≈24 h. These results indicate that most of the field-collected flies testing positive for sugar had sugar fed within the last 3 d, and those with visible blood had blood fed within the last 24 h.

In our field study, we did not observe a large difference in sugar feeding between male and female stable flies as did Jones et al. (1985). Although more females than males were observed with detectable sugar, the difference was <2%. The frequencies of sugar feeding among the different traps reflected the expected distribution of nectar sources. In the rural environment, more flowers are expected in pastures than croplands (primarily corn, sorghum, and wheat) or feedlot areas. Flower gardens were found throughout the urban environment. The seasonal distribution of sugar feeding also reflected the higher availability of nectar sources in the early season compared with midsummer. These results indicate that stable flies are opportunistic nectar feeders.

The percentage of the flies with visible blood in the gut was lower than expected, <1%. The bloodfeeding rate is much lower than that observed in Kansas by Guo et al. (1998). Those authors found 70–80% of flies captured on Alsynite sticky traps positioned adjacent to feedlot pens occupied by cattle showed visible evidence of blood in the gut. Scholl (1986) observed blood in the guts of only 2% of flies dispersing between feedlots; a number more similar to our observations. Two possible explanations for the small number of flies with blood are as follows: because the traps were collected every 24 h and our laboratory study indicated that most of the flies digested their blood between 24 and 48 h, some flies may have digested their blood meals after being caught on the traps and before the traps were collected and flies frozen. Second, Alsynite traps tend to collect younger, previtellogenic flies (Guo et al. 1998). This sampling bias and the trap locations may have reduced the number of bloodfed flies collected relative to the field population. The low

number of flies collected with visible blood was especially unexpected at the zoo where the trap was located adjacent to the pony rides. Stable flies were observed feeding on the ponies throughout the study period. Our results showed a small positive interaction between blood feeding and sugar feeding. This may reflect an increased chance for flies who have obtained supplemental energy through nectar to obtain a blood meal successfully.

In our laboratory study, we were able to see blood in the gut of between one third and one half of the stable flies blood feeding 24–48 h earlier. If we conservatively predict that we could detect blood in one third of the wild flies captured by Alsynite sticky traps that had blood fed within the last 24 h, we can estimate the overall bloodfeeding rate to have been 2.5% ($3 \times 0.8\%$) or less during the previous 24 h. Similarly, in the laboratory, we were able to detect sugar in 95% of the flies at 24 h, 80% at 48 h, and 40% at 72 h. Assuming that the number of flies in each of those three classes was collected equally on the traps, we can calculate that $\approx$40% of the observed sugar-positive flies had fed on sugar during the last 24 h and the others fed on sugar $>$24 h previously. Hence, overall, 5% (40% of 12.1%) or more of the field-collected stable flies had fed on sugar during the last 24 h. Based on these estimates, conservatively biased toward increasing the bloodfeeding rate and reducing the sugar feeding rate, stable flies within the cohort attracted to, and collected on, Alsynite traps are sugar feeding at twice the rate of blood feeding. Sugar feeding is clearly an important aspect of stable fly biology and one that has not been adequately exploited in studies of stable fly biology and for the development of control technologies.

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