

## COLLECTION OF BLOOD-ENGORGED BLACK FLIES (DIPTERA: SIMULIIDAE) AND IDENTIFICATION OF THEIR SOURCE OF BLOOD

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**ABSTRACT.** Blood-engorged black flies were captured by 2 methods: early morning application of a quick knock down insecticide (resmethrin) into tree canopies, and late afternoon to early evening truck trapping. More engorged flies were captured per man-hour with the tree spraying method than with the truck trap. The percentages of females captured by the spraying and truck trap methods that were engorged were 18 and 1.3, respectively. The most commonly engorged species captured were *Prosimulium magnum* and *P. mixtum/fuscum*, and *Simulium jenningsi* and *S. venustum* (70% of total). Blood sources of 12 black fly species were identified. Mammals comprised 95% of the blood sources identified. The most common hosts were equines (31%), bovines (25%) and raccoons (19%). Seven percent of all meals identified were from mixed sources, of which 43% involved both mammals and birds.

### INTRODUCTION

Serologic identification of blood sources in wild caught hematophagous insects is the best way to quantitatively assess their natural blood-feeding patterns (Washino and Tempelis 1983). Few such studies have been published on black flies because of difficulty in capturing newly engorged females (Davies et al. 1962, Disney and Boreham 1969, Walsh 1980, Roberts and Irving-Bell 1987). Most host-feeding studies on simuliids have been conducted by trapping flies using various animals as bait or by collecting engorging females from wild or domestic animals (Davies and Peterson 1956, Anderson and DeFoliart 1961, Fallis 1964).

Light traps (Davies et al. 1962), suction traps (Johnson et al. 1982), truck traps (Davies and Roberts 1973, Roberts and Irving-Bell 1985, 1987), colored sticky panels (Walsh 1980) and other sticky traps (Bellec and Hebrard 1984) are methods that have been used in attempts to capture engorged black flies away from hosts. No completely passive method of capturing engorged females at rest has been successfully employed. Service (1977) proposed spraying insecticides in tree canopies, which is where Wolfe and Peterson (1960) suggested nearctic black flies rest.

The present study was conducted to evaluate the usefulness of two relatively unbiased adult collection techniques (insecticide spraying and truck trapping) for capturing blood-engorged black flies in the northeastern United States.

This paper presents the results of those efforts and the identity of blood sources from the modest sample of engorged black flies obtained during the course of this evaluation.

### MATERIALS AND METHODS

**Truck trap:** The truck trap was constructed of 0.25-mm mesh nylon netting and aluminum angle stock. The trap was held on the roof of the truck cab with suction cups and gutter straps. The trap opening was 1.3 m wide  $\times$  1 m high. The cone-shaped net was 2 m long with a 4-cm opening at the tip of the cone. Insects were collected in 4 cm diam  $\times$  30 cm long plastic vials with bottoms replaced with nylon net. Vials were secured in the tip opening by Velcro. Vials could be removed from the net, capped and replaced with an empty one within a few seconds after the vehicle stopped.

**Truck trapping:** Truck trapping was conducted on two, 6.5 km routes, hereafter called A and B, in the towns of Leverett and Wendell in Franklin Co., MA. Both routes were along heavily forested rural roads bordering streams, beaver ponds, a lake, numerous human residences and 2 horse and one cattle farm.<sup>3</sup> The truck was driven at approximately 29 km/h. Vials were changed at ca. 1.6 km intervals. Actual distance depended on habitat. In 1980, route A was sampled hourly between 0400–2200 h once each week during July and August. During 1981, both routes A and B were sampled hourly between 0600–2200 h once each week from late April through June. In 1982, route A was sampled weekly between 1400 and 2200 h from late April

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until mid-September. Captured black flies were stored on ice until returned to the laboratory where they were counted, identified and engorged females frozen at  $-40^{\circ}\text{C}$  until blood meal identification.

**Insecticide spraying:** Insecticide spraying was done at the edge of a horse pasture on route A. The lower canopy of maple and pine trees near a stream at the edge of the pasture was sprayed with resmethrin by backpack sprayer (Kioritz model DM 9) equipped with a ULV nozzle calibrated to deliver 54 sec mineral oil at 200 ml/min. Resmethrin was diluted to a concentration of 3.3% with 54 sec mineral oil. Trees were sprayed for 5 min between 0400 and 0600 h, generally when it was too dark and cool for substantial black fly activity and no host-seeking activity was observed at ground level. After spraying, trees were shaken as much as possible to knock dead flies retained within the foliage onto king-size white bed sheets ( $269 \times 274$  cm) spread on the ground beneath the trees. Engorged black flies were identified to species and frozen until blood meal identification. Several different pairs of trees were sprayed on 16 different days between late May and mid-July 1980.

**Blood meal identification:** Blood meals were identified by the modified capillary precipitin technique (Edman 1971, Tempelis and Lofy 1963). Engorged flies were individually crushed in 3.5 ml polyethylene centrifuge tubes containing 0.4 ml of 0.85% phosphate buffered saline at pH 7.2 and allowed to extract overnight at  $2.5^{\circ}\text{C}$ . Extracts were centrifuged and the supernatants transferred to clear polycarbonate 3.5 ml tubes using a Pasteur pipette. Each extract was first

tested with broadly reacting avian and mammalian antisera. Extracts which did not react with either antiserum were then tested with reptile and amphibian antisera. Once the vertebrate class of the meal was established, the extract was tested with the appropriate specific antisera. In the case of mammal blood meals, we tested for rabbit, ruminant, equine, human, porcine, raccoon, opossum, scurid rodent, striped skunk and canine; for avian blood meals we tested for passerine, ciconiiform, gruiiform and chicken.

## RESULTS

**Sampling:** A total of 124 engorged black flies representing 3 genera and 12 species were collected by the truck trapping and tree spraying techniques described above (Table 1). Engorged females of 12 species were collected with the truck trap compared with 3 species by tree spraying. The only species collected by tree spraying not captured by truck trapping was *Simulium vernum* Macquart. Eighteen percent of females captured by tree spraying were engorged, compared with 1.3% by truck trapping (Table 1). We estimate that 385 h were devoted to truck trap sampling and 32 h to sampling with the backpack sprayer. This calculates to 0.28 and 0.47 engorged flies per hour of sampling effort for the truck trap and tree spraying methods, respectively.

Eighty-four percent of the female black flies collected by tree spraying were from pine trees (*Pinus* spp.), and 16% were from maple trees (*Acer* spp.). Of the 15 engorged females captured

Table 1. Blood engorgement of female black flies captured by truck trap and insecticide spray.

Species	Truck trapping <sup>1</sup>		Tree spraying <sup>2</sup>	
	No. captured	No. engorged	No. captured	No. engorged
<i>P. mixtum/fuscum</i>	3,501	14	14	0
<i>P. magnum</i>	514	18	2	1
<i>St. mutata</i>	2,024	8	8	0
<i>S. venustum</i>	930	26	38	8
<i>S. verecundum</i>	210	11	0	—
<i>S. tuberosum</i>	133	5	0	—
<i>S. vittatum</i>	91	2	0	—
<i>S. jenningsi</i> <sup>3</sup>	1,263	20	7	1
<i>S. gouldingi</i>	27	3	0	—
<i>S. aureum</i>	20	1	0	—
<i>S. croxtoni</i>	17	1	0	—
<i>S. vernum</i>	0	—	15	5
Total (%)	8,730	109 (1.3)	84	15 (17.9)

<sup>1</sup> Combined truck trap catches for 1980, 1981 and 1982. Represents 450 man-hours of work or ca. 1 blood meal/4 h of effort.

<sup>2</sup> Total of 32 trees sampled on 16 different days in 1980. Represents 32 man-hours of work or ca. 2 blood meals/4 h of effort.

<sup>3</sup> Includes members of *S. jenningsi* complex and *S. fibrinflatum*.

by spraying, 14 were from pine trees and one was from a maple tree.

**Blood meal sources:** The 123 engorged black flies tested consisted of 12 species from 3 genera. Of these, 105 (85%) reacted at least in screening tests with mammal and avian antisera (Table 2). None reacted with the reptile or amphibian antisera. The majority of meals identified were mammal except for meals tested from *Simulium gouldingi* Stone and *S. aureum* Fries.

Specific blood sources of identified meals are summarized in Table 2. For all black fly species combined, 31% were from equines, 25% from bovines, 19% from raccoons, 13% from unidentified mammals and 5% from birds. Seven percent of all meals were from more than one host group. One *Prosimulium mixtum/fuscum* Syme and Davies meal was from a dog. The only human meal identified was from *S. vernum*. *Stegopterna mutata* (Malloch) and *S. gouldingi* each had one blood meal identified as passerine. Only 1 engorged *S. aureum* was captured; it had fed on a ciconiiform bird.

Table 3 is a summary of the mixed meals identified. *Prosimulium magnum* Dyar and Shannon, *St. mutata*, *Simulium croxtoni* Ni-

cholson and Mikel, *S. vernum* and *Simulium venustum* Say contained blood from more than one host. Raccoons were involved in 4 of the mixed meals. *Prosimulium magnum*, *St. mutata* and *S. venustum* had mixed meals involving both avian and mammal blood (Table 3).

Five of the 6 engorged *S. venustum* captured by tree spraying were identified as having fed on a horse, and the other one on both horse and bovine blood. Engorged *S. venustum* captured by truck trap had a wider variety of blood sources. They included meals on 6 unidentified mammals, 6 horses, 5 bovines, 5 raccoons, and 1 raccoon/bovine combination. Of the 6 *S. venustum* that had fed on raccoon, all were collected from sections of the truck trap route that were near water. The same was true for the other species that had fed on raccoons (Table 2).

### DISCUSSION

Spraying trees with resmethrin was about twice as efficient as the truck trap in terms of man-hours spent capturing blood-engorged black flies. It is also highly efficient compared with other techniques that have been reported for capturing blood-engorged black flies (John-

Table 2. Blood sources of engorged black flies collected in spring and summer, 1980-82, in Franklin Co., MA.

Species	No. tested/ no. reacting	Host blood source identified										
		Mammal							Avian		Mam./Avian	
		Bov.	Equ.	Rac.	Dog.	Hum.	Mixed	Unid.	Pass.	Cicon.	Unid.	Mixed
<i>P. magnum</i>	19/17	6	5	3	0	0	1	1	0	0	0	1
<i>P. mixtum/fuscum</i>	13/12	3	2	4	1	0	0	2	0	0	0	0
<i>St. mutata</i>	7/6	1	2	1	0	0	0	0	1	0	0	1
<i>S. gouldingi</i>	3/3	0	0	1	0	0	0	0	1	0	1	0
<i>S. vernum</i>	7/7	1	2	1	0	1	0	1	0	0	0	1
<i>S. aureum</i>	1/1	0	0	0	0	0	0	0	0	1	0	0
<i>S. croxtoni</i>	1/1	0	0	0	0	0	1	0	0	0	0	0
<i>S. jenningsi</i>	20/15	4	6	3	0	0	0	2	0	0	0	0
<i>S. tuberosum</i>	5/4	2	2	0	0	0	0	0	0	0	0	0
<i>S. verecundum</i>	11/9	4	3	2	0	0	0	0	0	0	0	0
<i>S. venustum</i>	34/29	5	11	5	0	0	2	6	0	0	0	0
<i>S. vittatum</i>	2/1	0	0	0	0	0	0	1	0	0	0	0
Total	123/105	26	33	20	1	1	4	13	2	1	1	3
(Percent)		(24.8)	(31.4)	(19.0)	(0.9)	(0.9)	(3.8)	(12.4)	(1.9)	(0.9)	(0.9)	(2.9)

Table 3. Identification of blood sources in mixed blood meals.

Mixed meal sources	Black fly species				
	<i>P. magnum</i>	<i>St. mutata</i>	<i>S. croxtoni</i>	<i>S. vernum</i>	<i>S. venustum</i>
Bovine/equine	1	—	—	—	1
Bovine/raccoon	—	—	—	—	1
Equine/raccoon	—	—	1	—	—
Raccoon/passerine	1	1	—	—	—
Equine/ciconiiform	—	—	—	1	—

son et al. 1982, Lamontellerie 1967, Walsh 1978, 1980; Service 1979, Bellec and Hebrard 1984). The exception is the transparent screen sticky trap method reported by Bellec and Hebrard (1984). Twenty-one percent of the flies captured in their study were engorged (vs. 18% by tree spraying in our study). The tree spraying technique has the advantage of being applicable in areas where truck trapping would not be possible. Previous studies with truck traps in England and Africa reported much higher engorgement rates than we observed (Davies and Roberts 1973, Roberts and Irving-Bell 1985, 1987). However, these studies were conducted in open savanna and farmland habitats where flies were perhaps captured in route from their hosts to a suitable resting site.

The high percentage of engorged females captured by the knockdown method supports Wolfe and Peterson's (1960) hypothesis that many black flies rest in tree canopies. Males and teneral, gravid and empty females also were captured by this method. We have sampled understory vegetation, grasses along stream banks and in fields, and leaf litter with both power aspirators (Nasci 1981) and sweep nets during the day and at night and have captured no engorged females and very few males or unengorged females. The only time when large numbers of flies (particularly *P. mixtum/fuscum* and *Simulium decorum* Walker) were found along stream banks was just after eclosion or when flies were congregating prior to mating or oviposition. These findings do not agree with several previous studies which suggest that Nearctic black flies rest in understory vegetation or along stream banks (Service 1977).

Most of the engorged (94%) and non-engorged (82%) black flies captured by tree spraying were from the pine trees. This may have been due to better dispersal of the insecticide in the canopy of pines, better knockdown of flies through the branches to the ground, a preference to rest in pine over maple, or better location of the pine trees within the general habitat. Much comparative work remains to be done on this aspect. The efficiency of the method should be greatly enhanced once the characteristics of prime aerial resting sites are known. Methods also need to be developed to distribute the insecticide higher into the tree canopy. Preliminary evidence suggests that the maximum vertical killing distance of the spray was about 8 meters. The use of a tree crane to spray downward from above the canopy would likely be more efficient in areas that are accessible to such heavy equipment.

We did not collect and identify sufficient blood meals of any one black fly species to draw

firm conclusions about feeding patterns or preferences. Our results agree in general with the findings of the limited previous studies on Nearctic black flies. (Davies and Peterson 1956, Anderson and DeFoliart 1961, Fallis 1964). Large mammals, particularly bovines and horses, were common hosts. Horses were probably the most abundant host near our study site and the highest percentage of meals were from this host (Table 2). Downe and Morrison (1957) found that *S. venustum* fed more frequently on horses than on cattle, pigs or chickens housed together in a one-room barn. The bovine feeding rate we observed also was high. Although our bovine antiserum did not distinguish cattle blood from other ruminants, the numbers of sheep, goats and white-tailed deer were limited, compared with cattle.

*Simulium venustum* and *P. mixtum/fuscum* are the major early season black fly species that annoy and bite humans in Massachusetts and throughout much of North America (Davies and Peterson 1956, Davies et al. 1962, Cupp and Gordon 1982). No human meals were identified from these black flies. Despite their numbers, humans are probably not important blood sources of *S. venustum* or *P. mixtum/fuscum* in our area. Moreover, few female *S. venustum* attracted to humans are observed to actually feed (Schreck et al. 1980, Simmons 1985). In comparison, *P. mixtum/fuscum* aggressively attack humans in this area (Simmons 1985) but humans are unlikely to tolerate much engorgement. The only species with a blood meal of human origin was *S. vernum* (Table 3). Davies et al. (1962) found that in Scotland this species (as *Eusimulium latipes*) fed on birds, humans and other mammals. There are other reports of this species feeding on humans as well (Davies and Peterson 1956, Fallis 1964). As discussed by Davies et al. (1962), this species does not follow the pattern of other *Eusimulium* species which generally are believed to be strict bird feeders (Fallis and Bennett 1958). Though widely distributed, and fairly abundant near our study sites, we have never collected *S. vernum* biting humans; but it has been collected in overhead net samples (Simmons 1985).

Twenty percent of the identified blood meals were from raccoon (Table 3). Eight species were found to have fed on raccoons. Four species had raccoon blood in mixed meals. (Table 3). There are 2 previous reports of *S. venustum* feeding on raccoons (Davies and Peterson 1956, Wright and DeFoliart 1970), but none of the 7 other species were previously shown to feed on raccoons. Raccoons are nocturnal foragers, particularly near water; and most black flies are diurnal (Wenk 1981). Perhaps black flies feed

on raccoons as they rest in open tree cavities near streams during the daytime.

Seven percent of all meals were from mixed blood sources (Table 3). This may be related to host defensive behavior (Simmons 1985). Defensive behavior has been shown to cause feeding disruption in mosquitoes and to contribute to multiple feeding (Edman et. al. 1985).

*Simulium venustum* is a potential vector of *Dirofilaria* spp. in Massachusetts. Between 1 and 2% of the parous *S. venustum* females captured in truck trap collections were found to have L1 and L2 filaria in the Malpighian tubules. Addison (1980) reported that *S. venustum* is a vector of *Dirofilaria ursi* Yamaguti of black bears in Canada. Bears are rare in the vicinity of the study sites (Wendell Dodge, personal communication). Dog heartworm is endemic in the area of the study sites (Paul Katz, personal communication). Preliminary studies in which *S. venustum* were fed on a heartworm-infected dog resulted in destruction of Malpighian tubules, but no L2 or L3 larvae were recovered (Simmons and Edman, unpublished data). Raccoons may be a possible source of the infection. They are the host of 2 filarial species known to develop in the Malpighian tubules of certain mosquitoes (Hawkins and Worms 1961).

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