

Table 1. Distribution of *Amblyospora*-infected oenocytes in adult female *Culex salinarius*.

Age in days (Post-emergence)	Mean number of oenocytes/body regions								
	Head	Thorax	Abdominal segments						
			I	II	III	IV	V	VI	VII-VIII
4-7 (n=8)	0.9	15.9	3.0	1.8	2.9	2.8	3.1	3.1	2.0
10-13 (n=1)	0.3	14.4	1.1	2.7	2.1	2.5	3.2	2.7	0.5
18-21* (n=9)	0.7	12.3	2.7	2.7	2.3	1.3	0.8	2.4	0.9

\* Fed on blood 77 hr prior to fixation.

because of the inability to determine the frequency of fusion of the oenocytes into syncytia in the different body segments and the fact that the hemocoel compartments of the various body regions are not comparable in volume. The data are presented merely to demonstrate the wide distribution of the oenocytes in adult females of different ages, and that there is no marked difference in distribution in blood-fed mosquitoes.

The microsporidia have a specialized tubular structure, the polar filament, which is coiled within the spore. Under appropriate conditions the polar filament is forcibly extruded, often penetrating the host tissue, and the sporoplasm then exits the spore through the polar filament and enters the host tissue. Andreadis and Hall (1979b) reported that a very high percentage (ca. 90% over 5 gonotrophic cycles) of the progeny of an infected *Cx. salinarius* female are infected. The exact mechanism of infection of the developing oocytes is not known. For the oocytes to be penetrated directly by the extruded polar filaments, the oenocytes would have to be clustered around the ovaries. This has been suggested as one possible mechanism for infection of the oocytes (Andreadis and Hall 1979a). Although oenocytes were sometimes observed adjacent to the ovaries in the present study, this occurrence did not seem to be sufficiently common to account for infection of most of the developing oocytes. There is certainly no preferential migration of the infected oenocytes to the ovaries in blood-fed *Cx. salinarius* as reported for *P. anophelis*-infected oenocytes in *An. quadrimaculatus*.

An alternative hypothesis for oocyte infections is that the spores extrude their polar filaments and release the sporoplasms into the hemolymph. The sporoplasms could then be carried to the ovaries. Extensive electron microscopic studies of mosquitoes during the period 72-96 hr after a blood meal will be required to elucidate the exact mechanism of infection of the oocytes.

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#### EVALUATION OF A PORTABLE CO<sub>2</sub> GENERATOR FOR SAMPLING BLACK FLIES

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Black fly attacks on cattle are a serious problem in Alberta and Saskatchewan, Canada, affecting beef and milk production, decreasing the calving rate and causing death (Fredeen 1977, Haufe and Croome 1980). Permethrin, registered in Canada for on-farm control of adult black flies, protects cattle for only 10 days (Shemanchuk 1981). Because black fly attacks can occur any time from June through September, accurate monitoring of the adult black fly population is useful for predicting when to apply chemical treatments to cattle.

The dry ice-baited silhouette trap has been shown to be an effective method for sampling populations of adult black flies (Shipp 1985). This trap, however, must be examined daily to

replenish the dry ice bait, which is not always readily available in rural areas.

A carbon dioxide generator which emits CO<sub>2</sub>, heat and moisture has been developed for use with light traps in sampling mosquito populations (Armatron International Inc., Melrose, MA). This generator is portable and uses a platinum catalyst that emits CO<sub>2</sub> at an approximate rate of 240 ml min<sup>-1</sup>. The generator can be operated from a 0.5 kg propane tank for 88 hours. A 6-volt dry-cell battery is used as a power source to ignite the catalyst. An advantage of the generator over dry ice for monitoring black flies is that the generator is compact (1.5 kg) and, therefore, can be used in remote and rural areas. Also, the generator does not require daily servicing and cost of propane usage per day is considerably less than that of dry ice (\$0.30 vs \$2.55).

The purpose of the present study was to evaluate the effectiveness of the CO<sub>2</sub> generator as a source of CO<sub>2</sub> for a baited silhouette trap. The evaluation was determined by comparing the effectiveness of silhouette traps provided with a CO<sub>2</sub> generator with that of silhouette traps baited with and without dry ice.

The study was conducted on a 20-ha pasture near Grassland, Alberta in 1984. Traps were evaluated on 12 days between June 27 and September 13. No cattle were present in the pasture during the study. The experimental design consisted of 3 randomized blocks, each having a north-south 100-m transect containing 1 trap of each type, spaced 50 m apart. On each trap day, trapping was conducted between 5 hr after sunrise and sunset. Trap collections were emptied at the end of the sampling period and preserved in 95% ethanol. Female black flies were identified to species using keys by Peterson (1960) and Fredeen (1981).

The silhouette trap has been described previously by Shipp (1985). For the 2 baited traps, either a block of dry ice (3.0-3.5 kg) wrapped in paper was suspended just beneath the body of the trap, or the orifice for the CO<sub>2</sub> generator was placed at the same height. To estimate the CO<sub>2</sub> output from the dry ice in the field, the

rate of CO<sub>2</sub> output from a 3.0-kg block of dry ice wrapped in paper was determined in the laboratory for a constant temperature and humidity using the gas constant equation (Weast 1972). The CO<sub>2</sub> output at 15, 20 and 25°C and 60% relative humidity was 1888, 2281 and 2794 ml min<sup>-1</sup>, respectively. The CO<sub>2</sub> output from the generators used in this study ranged from 264-297 ml min<sup>-1</sup>.

Eight species of black flies were collected using the 3 trap types (Table 1). The dry ice-baited silhouette trap sampled 21 times more black flies than the CO<sub>2</sub> generator-baited silhouette trap and 38 times more flies than the unbaited silhouette trap.

Table 1. Total number of adult female black flies (*Simulium* spp.) sampled by baited (dry ice or CO<sub>2</sub> generator) and unbaited silhouette traps over 12 days near Grassland, Alberta, 1982.

Species	Silhouette trap			Total
	Dry ice	CO <sub>2</sub> generator	Unbaited	
<i>S. arcticum</i>	29,778	756	234	30,768
<i>S. verecundum</i>	3,563	671	527	4,761
<i>S. decorum</i>	600	146	81	827
<i>S. vittatum</i>	250	67	49	366
<i>S. venustum</i>	135	24	27	186
<i>S. vernum</i>	113	5	0	118
<i>S. meridionale</i>	60	4	0	64
<i>S. aureum</i>	28	3	1	32

For 5 of the most numerous species trapped (*Simulium arcticum* Malloch, *S. verecundum* Stone and Jamnback, *S. decorum* Walker, *S. vittatum* Zetterstedt and *S. venustum* (Say)), data were summed over the sampling period for each kind of trap, transformed to log mean, and analyzed using analysis of variance. A multiple mean comparison procedure (Tukey's Test) was used to determine significant differences ( $P < 0.05$ ) among methods for sampling black fly populations (Steel and Torrie 1960). Trap type x day interaction was investigated for *S. arcticum* and *S. verecundum* to determine if the trend of

Table 2. Comparison of baited (dry ice or CO<sub>2</sub> generator) and unbaited silhouette traps for sampling 5 black fly species (*Simulium* spp.) near Grassland, Alberta, 1982.

Silhouette trap	Species*				
	<i>S. arcticum</i>	<i>S. verecundum</i>	<i>S. decorum</i>	<i>S. vittatum</i>	<i>S. venustum</i>
Dry ice	9.16a	7.06a	5.29a	4.35a	3.81a
CO <sub>2</sub> generator	5.47b	5.38b	3.85ab	3.00a	2.18a
Unbaited	4.35c	4.96b	3.05b	2.37a	1.54a

\* Mean of log counts for all the sampling dates.

<sup>a-c</sup> Means within a column followed by the same letter are not significantly different at the 5% level (Tukey's multiple comparison test).

catch sizes for the different traps over time was significantly different ( $P < 0.05$ ).

The dry ice-baited silhouette trap sampled significantly greater numbers of *S. arcticum* and *S. verecundum* than any of the other traps examined (Table 2). The CO<sub>2</sub> generator trap sampled greater numbers of these two species than the unbaited silhouette trap. The trap type x day interaction was significant for *S. arcticum* and *S. verecundum*. For *S. decorum*, the dry ice-baited silhouette trap sampled a significantly greater number of flies than the unbaited silhouette trap. There were no significant differences in the numbers of *S. vittatum* and *S. venustum* caught in any of the traps. Only 366 *S. vittatum* and 186 *S. venustum* were collected over 12 days.

*Simulium arcticum* is the major black fly pest species of cattle in Alberta (Fredeen 1969). Traditionally, control programs for black flies in Alberta have centered around monitoring population changes for *S. arcticum*. The CO<sub>2</sub> generator-baited silhouette trap collected fewer *S. arcticum* than the dry ice-baited trap, but more *S. arcticum* than the unbaited silhouette trap. However, the trends in the numbers of *S. arcticum* over time were significantly different between the dry ice- and CO<sub>2</sub> generator-baited silhouette traps. In this study, the CO<sub>2</sub> generator was not as effective as dry ice for monitoring black fly populations using the silhouette trap. However, the CO<sub>2</sub> generator could be useful in remote areas where dry ice is not available.

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#### TECHNIQUE FOR VOLUMETRICALLY MEASURING EGGS OF *CULEX QUINQUEFASCIATUS*

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It has been demonstrated that eggs of some *Anopheles* species can be dried and volumetrically measured (Dame et al. 1978, Bailey et al. 1979). This development greatly increased the efficiency of mass rearing *Anopheles albimanus* Weidemann in El Salvador (Bailey et al. 1980). In the past this method has not been used with *Culex* species because mosquitoes of this genus lay their eggs in rafts. This paper reports the development of a suitable technique for drying egg rafts of *Culex quinquefasciatus* Say, thus allowing volumetric egg measurements to be made for setting rearing trays.

Freshly collected egg rafts, not more than 24 hr old, were placed in a polyethylene cup 10 cm diam. with the bottom replaced with organdy cloth for drying in an apparatus previously described by Dame et al. (1978). Immediately after drying for ca. 20 min, the rafts were transferred to a 120-ml empty vial and individual eggs were separated by lightly pressing the rafts against the wall of the vial with an artist's brush (#4 Wilton Flat, Windsor, NJ). The eggs were then sifted through a screen (20 mesh/cm insert in a modified bottle cap) and volumetrically measured into graduated pipettes through a funnel. To determine if this handling technique caused any adverse effects on further development, the eggs were placed in rearing trays (56 × 43 × 7.5 cm) containing 3 liters of water and the larvae were reared to the pupal stage using procedures similar to those reported by Dame et al. (1978) in their paper on rearing *An. albimanus*. Pupal harvests were made on days 5 and 6 after trays were set using the ice water technique described by Hazard (1967). Reported here are the results of 3 replicates, each of which included 3 trays at 3 test densities of dried eggs (0.04, 0.05 or 0.06 ml per tray). Controls consisted of 10 nondried, intact egg