THE EFFICIENCY OF VARIOUS COLLECTION TECHNIQUES FOR SAMPLING CULEX ANNULIROSTRIS IN SOUTHEASTERN AUSTRALIA

RICHARD C. RUSSELL

School of Public Health and Tropical Medicine, University of Sydney, New South Wales, 2006, Australia

ABSTRACT. Dry-ice (CO_2) baited EVS (Encephalitis Virus Surveillance) light traps collected significantly more *Culex annulirostris* than unbaited EVS or CDC light traps, chicken-, guinea pig- and rabbit-baited EVS traps, or cubic foot resting boxes. Results from the animal-baited traps indicated a preference for bird versus mammal bait. Females sampled by the resting boxes had significantly lower parity rates than females sampled by dry-ice baited EVS traps. Parity rates were generally similar among females sampled by dry-ice baited EVS traps, unbaited CDC light traps and animal baited EVS light traps.

INTRODUCTION

The mosquito *Culex annulirostris* Skuse is an important vector of arboviruses from both bird and mammal reservoirs in Australia (Doherty 1974). Mosquito populations can be monitored to predict seasons when above average abundance can occur, and investigate the bionomics of the vector species that relate to its involvement in virus cycles. The use of different techniques may relate to the objectives of field investigations. This paper reports the results of a study at two sites in southeastern Australia, in which collecting techniques were compared to determine their relative efficiency for assessing the relative abundance, host response and parity of female *Cx. annulirostris*.

MATERIALS AND METHODS

The first study area was in the Kanyapella State Forest adjacent to the Kanyapella Basin wildlife area, situated on the floodplain near the junction of the Goulburn and Murray rivers, 12 km east of Echuca in northcentral Victoria. Within the wildlife management area were artificial ponds maintained for waterfowl, which were a major larval habitat for mosquitoes, particularly Cx. annulirostris. The second study area was in a dry sclerophyll forest adjacent to Brian Road, Appin, New South Wales, approximately 70 km southwest of Sydney. The area has been partially cleared for grazing, and there are empoundments with vegetated margins, which were larval habitats for mosquitoes particularly Cx. annulirostris.

During February 1980, field studies were initiated to compare collecting techniques including a dry-ice (500 gm) baited EVS (Encephalitis Virus Surveillance) light trap (Rohe and Fall 1979); an unbaited CDC light trap (Sudia and Chamberlain 1962); an unbaited EVS light trap; EVS traps modified to accommodate small animals (chicken, guinea pig or rabbit—all approximately 500 gm); 1 cu ft resting boxes [5 boxes were used in each comparison per EVS trap) similar to the model of Goodwin (1942)]. Traps were set in pairs 20 m apart across the prevailing breeze, and each test was replicated three times. The traps were situated approximately 250 m from the major larval habitats at Appin, and approximately 2 km from the larval habitats at Echuca.

Female Cx. annulirostris were counted and parity status was determined according to the dissection technique of Detinova (1962). When collections were large, randomly collected subsamples were dissected. Abundance data were analysed by paired t-test after square root transformation, and the proportion of parous females sampled were tested by Chi-square test.

RESULTS AND DISCUSSION

The dry-ice baited EVS trap collected the greatest numbers of Cx. annulirostris, but was almost ineffective if operated without dry-ice (Table 1). The CDC light trap collected significantly fewer adults than the dry-ice baited EVS at both Appin and Echuca (p < 0.05). The EVS trap also collected more Cx. annulirostris at both locations when baited with dry-ice than when baited with either a chicken or guinea pig. When the animal baits were compared, traps baited with a chicken were superior to traps baited with either a guinea pig (p < 0.01, p <0.05) or rabbit (p > 0.01, p < 0.05) at Appin and Echuca, respectively. There was no significant difference in collection sizes when traps were baited with either a guinea pig or rabbit. Resting boxes were far more successful for sampling female Cx. annulirostris at Appin than at Echuca, but they collected on average only 2-5% as many as the dry-ice baited EVS trap. Male Cx. annulirostris were regularly collected in the resting boxes of the above traps. The CDC trap occasionally collected males of the species, but the dry-ice and animal baited traps did not collect male Cx. annulirostris on any occasion.

Culex annulirostris responded more to traps baited with a chicken versus guinea pig at both

Sampling method	Appin			Echuca		
	Abundance ¹	No. dissected	Prop. parous	Abundance	No. dissected	Prop. parous
Dry-ice EVS	$\begin{array}{rrrr} 113.3* & (19.1) \\ 5.3* & (2.1) \end{array}$	200	0.58	972.3* (68.0)	200	0.88*
Unbaited EVS		16	0.69	6.0* (4.6)	18	0.55*
Dry-ice EVS	99.3* (23.9)	2 00	0.57	1060.0* (92.5)	200	0.69
CDC light trap	39.3* (21.7)	100	0.63	627.7* (85.5)	200	0.72
Dry-ice EVS	$\begin{array}{rrr} 103.7* & (19.5) \\ 46.7* & (5.9) \end{array}$	200	0.53*	706.3* (194.8)	200	0.70
Chicken EVS		100	0.65*	226.0* (21.9)	200	0.75
Dry-ice EVS	94.0* (15.1)	200	0.59	693.7* (173.7)	200	0.71
Guinea pig EVS	29.0* (8.2)	80	0.60	137.0* (27.5)	200	0.74
Chicken EVS	54.3* (14.6)	150	0.69	227.7* (59.4)	200	0.69
Guinea pig EVS	30.3* (13.0)	90	0.62	147.3* (41.6)	200	0.73
Chicken EVS	64.3 (14.2)	150	0.60*	192.3* (17.6)	200	0. 69
Rabbit EVS	47.7 (20.2)	100	0.47*	136.7* (24.0)	200	0.73
Guinea pig EVS	53.7 (6.7)	150	$0.63 \\ 0.58$	143.7 (44.4)	200	0.6 2*
Rabbit EVS	57.7 (4.2)	150		136.3 (17.5)	200	0.78*
Dry-ice EVS	127.3* (18.0)	200	0.70*	1027.7* (221.6)	200	0.76*
Rest-boxes (5)	31.0* (14.1)	90	0.43*	128.7* (26.2)	200	0.56*

 Table 1. Comparison of sampling techniques for determining the relative abundance and parity of Culex annulirostris at Appin, New South Wales and Echuca, Victoria, Australia February 1980.

¹ Mean number of mosquitoes per trap night (sample standard deviation).

* = significantly different (at least at the p<0.05 level) as tested by paired t-test after square root transformation for the abundance values and by Chi-square test for the proportion parous values.

Echuca and Appin, and similarly responded to traps baited with a chicken versus rabbit at Echuca. Comparisons of mammalian baits showed that female *Cx. annulirostris* responded equally to traps baited with either a guinea pig or rabbit at Echuca or Appin. Thus in this study *Cx. annulirostris* responded more to birds than to mammals as a possible bloodmeal source.

In southern Australia Cx. annulirostris has been reported to feed on a wide variety of animals (Dobrotworsky 1965, Lee et al. 1954, Reeves et al. 1954). The baits used in this study are not indigenous hosts, and may not necessarily represent host feeding patterns of Cx. annulirostris in southeastern Australia. The results from the present study indicate feeding patterns more associated with birds than mammals. and thus are at variance with the general conclusions of Kay et al. (1979) that Cx. annulirostris prefers mammal to bird hosts in Queensland (with no evidence of a seasonal shift in host preference), and Wright (1981) that rabbit baited traps collected more Cx. annulirostris than chicken or guinea pig baited traps in northern Western Australia. The latter author suggested that the larger size of the rabbits, with their relatively greater output of carbon dioxide, could have been responsible for the advantage (along with the habit of the chickens of ingesting captured mosquitoes). The use of similarly sized bait animals, and their isolation from the captured mosquitoes in the modified EVS trap was an attempt to overcome these problems in

the present investigation. However the CO_2 output from the dry-ice bait might have exceeded that from either animal, and may be responsible for the greater number of mosquitoes collected by the dry-ice baited EVS trap when compared to the animal baited traps.

Culex annulirostris females must be parous to be potentially infective for arboviruses, so estimating proportion parous in a population is epidemiologically important. The proportion parous of a mosquito population can be used to estimate population survival rates and thus relates to potential to survive to an infective age for transmitting arboviruses. Female Cx. annulirostris collected by dry-ice baited EVS traps exhibited marginally lower parity rates than females collected by the animal baited traps. Compared to a CDC light trap and guinea pig baited trap, the dry-ice baited EVS trap collected a slightly smaller (not significant) proportion of parous females at both locations. However, when compared to chicken baited traps, the dry-ice baited EVS trap collected a significantly (p < 0.01) lower proportion of parous females only at Appin. At Appin females collected at bird bait exhibited a higher parous rate than females attracted to either guinea pig (not significant) or rabbit bait (P <0.05). At Echuca, however, the mammal baited traps attracted a higher proportion of parous females than chicken baited traps. Parity among females sampled by mammal baited traps varied between locations. At Appin, there was no

significant difference between the proportions parous attracted by guinea pig or rabbit bait, but at Echuca the females attracted to the guinea pig bait had a significantly (p < 0.05) lower parity rate than those attracted to the rabbit bait. The resting boxes sampled females with significantly lower parity rates than dry-ice baited EVS traps at both Appin and Echuca (p < 0.05, p < 0.05 respectively).

Gillies (1974) discussed the problem of obtaining a representative sample for determining the physiological age of a mosquito population. and indicated that age composition of females in a sample may vary according to the method and time of capture, geographical location and physiological state of individual females. Simultaneous use of different sampling methods which operate upon different principles has been proposed by Bidlingmayer (1974) to obtain a more objective sampling of the population being investigated. It has been reported that CO₂-baited traps collect a significantly greater proportion of nulliparous females than light traps (Feldlaufer and Crans 1979, Magnarelli 1975), but a significantly higher proportion of parous females may be sampled by CO2-baited traps than by artificial resting shelters (Milby et al. 1983). However, Morris and DeFoliart (1969) reported variability in parity among different mosquito species sampled by light and CO₂-baited traps. Parous rates were higher among female Culex tarsalis Coquillett sampled by CO₂-baited CDC light traps than among females sampled by New Jersey light traps and resting boxes in California (Meyer et al. 1984). High parity rates have also been reported for Culex salinarius Coquillett sampled by CO2 trap (Slaff and Crans 1981). Epidemiologically, the host-seeking and parous component of a vector population is of most concern and individuals taken at CO2-baited traps are considered to represent the host-seeking population (Gillies 1980, Reisen et al. 1983).

The dry-ice baited EVS trap can be recommended as the superior collection method for female Cx. annulirostris in southeastern Australia, and this trap also representatively samples the parous component of the Cx. annulirostris population at least as well as the other methods tested. Thus the dry-ice baited EVS trap would be more suitable for collecting arbovirus pools than from CDC light traps or shelters.

ACKNOWLEDGMENTS

The late Mr. J. Dawson, formerly Health Surveyor for the Echuca City Council, suggested the Kanyapella site for this study, and access to the Appin site was with permission of the Macarthur Development Board. Thanks are also due to Assoc. Prof. G. Berry for statistical assistance and Assoc. Prof. J. H. Bryan for manuscript review.

References Cited

- Bidlingmayer, W. L. 1974. The influence of environmental factors and physiological stage on the flight patterns of mosquitoes taken in the vehicle aspirator and truck, suction, bait and New Jersey light traps. J. Med. Entomol. 11:119-146.
- Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance with special reference to some vectors of malaria. W.H.O. Monogr. Ser. 47:1-216.
- Dobrotworsky, N. V. 1965. The mosquitoes of Victoria. Melbourne Univ. Press, Carlton.
- Doherty, R. L. 1974. Arthropod-borne viruses in Australia and their relation to infection and disease. Progr. Med. Virol. 17:136-192.
- Feldlaufer, M. F. and W. J. Crans. 1979. The relative attractiveness of carbon dioxide to parous and nulliparous mosquitoes. J. Med. Entomol. 15:140-142.
- Gillies, M. T. 1974. Methods for assessing the density and survival of blood-sucking Diptera. Annu. Rev. Entomol. 19:345-362.
- Gillies, M. T. 1980. The role of carbon dioxide in host finding by mosquitoes (Diptera: Culicidae): A review. Bull. Entomol. Res. 70:525-532.
- Goodwin, M. H., Jr. 1942. Studies on artificial resting places of Anopheles quadrimaculatus. J. Natl. Malaria Soc. 1:93-99.
- Kay, B. H., P. F. L. Boreham and G. M. Williams. 1979. Host preferences and feeding patterns of mosquitoes (Diptera: Culicidae) at Kowanyama, Cape York Peninsula, northern Queensland. Bull. Entomol. Res. 69:441-457.
- Lee, D. J., K. J. Clinton and A. K. O'Gower. 1954. The blood sources of some Australian mosquitoes. Aust. J. Biol. Sci. 7:282-301.
- Magnarelli, L. A. 1975. Relative abundance and parity of mosquitoes collected in dry-ice baited and unbaited CDC miniature light traps. Mosq. News 35:350-353.
- Meyer, R. P., R. K. Washino, T. L. McKenzie and C. K. Fukushima. 1984. Comparison of three methods for collecting adult mosquitoes associated with rice field and irrigated pasture habitats in northern California. Mosq. News 44:315–320.
- Milby, M. M., W. K. Reisen and W. C. Reeves. 1983. Intercanyon movement of marked *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 20:193–198.
- Morris, C. D. and G. R. DeFoliart. 1969. A comparison of mosquito catches with miniature light traps and CO_2 -baited traps. Mosq. News 29:424–426.
- Reeves, W. C., E. L. French, E. N. Marks and N. E. Kent. 1954. Murray Valley encephalitis: a survey of suspected mosquito vectors. Am. J. Trop. Med. Hyg. 3:147-159.
- Reisen, W. K., M. M. Milby, W. C. Reeves, R. P. Meyer and M. E. Bock. 1983. Population ecology of *Culex tarsalis* (Diptera: Culicidae) in a foothill environment of Kern County, California: temporal changes in female relative abundance, reproductive

status, and survivorship. Ann. Entomol. Soc. Am. 76:800-808.

- Rohe, D. L. and R. P. Fall. 1979. A miniature battery powered CO₂ baited light trap for mosquito borne encephalitis surveillance. Bull. Soc. Vector Ecol. 4:24-27.
- Slaff, M. and W. J. Crans. 1981. The activity and physiological status of pre- and posthibernating

Culex salinarius (Diptera: Culicidae) populations. J. Med. Entomol. 18:65-68.

- Sudia, W. D. and R. W. Chamberlain. 1962. Battery powered light trap, an improved model. Mosq. News 22:126-129.
- Wright, A. E. 1981. Ord River arboviruses—mosquito captures during 1976/77. J. Aust. Entomol. Soc. 20:47-57.

VIRGINIA MOSQUITO CONTROL ASSOCIATION 1848 Pleasant Ridge Road Virginia Beach, Virginia 23457

President: D. L. Cashman 2nd Vice President: Wilson Garland Secretary-Treasurer: Harry W. West

The VMCA has aided mosquito control agencies in Virginia since 1947.