ENVIRONMENTAL FACTORS AFFECTING MORTALITY OF ADULT *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) IN THE LABORATORY

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ABSTRACT. The effects of several environmental factors on mortality of adult *Culicoides variipennis* in the laboratory were evaluated. Daily mortality rates significantly increased when adult midges were maintained at an elevated constant temperature (26°C). Adult *C. variipennis* handled the least during routine maintenance procedures had the lowest daily mortality rate. Survival was not significantly affected by varying density levels of adult midges in the size of container routinely used in our laboratory. Implications of these observations using adult *C. variipennis* for future studies are discussed.

INTRODUCTION

The biting midge, *Culicoides variipennis* (Coquillett), is the primary vector of bluetongue virus (BTV) in sheep, cattle and wild ruminants, and of epizootic hemorrhagic disease virus in deer and cattle in the U.S.A. Bluetongue disease is a serious economic threat to domestic ruminants. It causes mortality in sheep, reproductive impairment in both sheep and cattle, and restrictions in the international movement of breeding livestock and germplasm (Gibbs and Greiner 1988, Holbrook 1988).

Procedures have been developed for mass rearing *C. variipennis* to conduct entomologic studies, to evaluate this insect as a vector of several disease agents of veterinary importance and to develop insect control strategies at the Arthropod-borne Animal Diseases Research Laboratory (Jones 1960, 1964, Jones et al. 1969, Akey et al. 1978, G. J. Hunt, unpublished data). However, similar rearing procedures have proved inadequate for maintaining many field-collected populations of immature or adult *C. variipennis*. Adults of these populations have survived poorly during extended holding periods, such as the extrinsic incubation period (10–14 days) required for BTV transmission studies. Excessive mortality has been observed with various species of field-collected *Culicoides* during vector competence studies (Jones et al. 1983, Mullen et al. 1985). Environmental factors (i.e., insect-rearing procedures) may contribute to high mortality in these natural populations of *C. variipennis* brought into the laboratory.

Of environmental factors which influence important life history traits of field-collected and laboratory-reared *C. variipennis*, only temperature and insect density during larval development have been studied in detail. Wing length in female *C. variipennis* has been shown to be inversely proportional to rearing temperature (Hensleigh and Atchley 1977, Mullens and Rutz 1983, Vaughan and Turner 1987). Akey et al. (1978) showed that adult size in female *C. variipennis*, as measured by dry weight and wing length, was inversely proportional to both larval density and rearing temperature. In addition, larger female adults lived longer and produced more eggs than did smaller adults. Mullens (1987) observed a seasonal trend of adult size in female *C. variipennis*, with an apparent inverse relationship between wing length and air temperature near the insect's natural habitat. The rate of development of immature *C. variipennis* has also been inversely related to temperature (Akey et al. 1978, Mullens and Rutz 1983, Vaughan and Turner 1987).

In the present study environmental factors, such as temperature, blood feeding, adult density per container, and insect-handling techniques, which might affect the mortality of adult *C. variipennis* in the laboratory, were evaluated.

MATERIALS AND METHODS

Test insects: Culicoides variipennis were obtained from the AA colony (established December 1957 from Edwards County, Texas) (Jones 1960) and the AK colony (established August 1973 from Owyhee County, Idaho) (Jones and Foster 1978). Both colonies have been continually maintained without the addition of wild *C. variipennis* since establishment. Insect-rearing procedures for mass production were used (G. J. Hunt, unpublished data).

Experimental design: Male and female adult *C. variipennis*, 24- to 48-hr old, were randomly divided into two groups from each colony. Insects in the first group (A) were anesthetized with medical-grade CO_2 gas and placed in blood-feeding cages. Teneral female adults were allowed to feed on defibrinated sheep blood without additives through membranes prepared from the skins of 1- to 3-day-old chicks using an artificial blood-feeding apparatus (G. J. Hunt and C. N. McKinnon, unpublished data). After

2.5 h of feeding, insects were anesthetized and sorted by sex and degree of blood feeding on refrigerated tables with the aid of dissecting microscopes. One-hundred fully-engorged midges from each colony were placed in 0.24-liter (0.5-pint) cardboard ice cream containers with fine-mesh nylon organdy coverings. In each container, a moist substrate of absorbent cotton, filter paper and deionized water placed in a small dish was provided for oviposition, and a vial with a cotton dental wick containing 10% sucrose solution was alternately provided as a carbohydrate source for one day followed by deionized water for two days. Three containers (replicates) from each colony were maintained at constant temperatures of either 20 ± 1°C or 26 ± 1°C, 40–50% RH and a 13L:11D photoperiod. The oviposition substrates and cotton wicks were remoistened each day. On the fourth day, the oviposition dishes with eggs were replaced with empty dishes. The second group (B) of insects was processed and maintained in the same manner, except these teneral female adults were not anesthetized nor provided blood meals or oviposition substrates. For the third group (C) of insects, pupae from each colony were obtained two days prior to the initial setup of the first two groups of insects. Female pupae were sorted with the aid of a dissecting microscope and placed in 0.24-liter containers with organdy coverings. Each container accommodated 110 pupae (to compensate for an expected 91% adult-emergence rate) placed on a moist substrate of absorbent cotton and deionized water in a small dish for emergence. Three containers (replicates) from each colony were maintained under the same rearing conditions as with the first two groups of insects. On the second day, the emergence dishes were replaced with empty dishes. In summary, the varying handling techniques for the three groups of adult *C. variipennis* included anesthetizing, blood feeding, anesthetizing, chilling and sorting for group A; anesthetizing, chilling and sorting for group B; and nothing for group C.

The effect of varying densities of adult insects on mortality of *C. variipennis* in the 0.24-liter containers was also studied. Three containers (replicates) of 100, 75, 50 and 25 blood-fed females per container from the AA colony were processed in the same manner as those midges in group A and were maintained at 20 ± 1°C.

All containers in both experiments were examined daily; dead insects were counted, removed and discarded from each container. The daily mortality rate, the number of dead female adults divided by the total number of female adults present on that day, was determined in each container for 15 days.

Data analysis: Mortality rates and the $\log_{10}$ (x + 1) transformations for normality were analyzed by a three-way analysis of variance (ANOVA).

RESULTS

Only the results of the mortality rates of adult *C. variipennis* are presented in Table 1 since both ANOVA analyses showed the same results. Several environmental factors had a significant effect on daily mortality rates.

A significant difference was detected between the two constant temperatures for either colony (Table 1). Daily mortality was higher at 26°C than at 20°C (Fig. 1). Since no differences in mortality rates were detected between the two colonies, these results were combined and showed significant differences among the three handling techniques (Table 1). The highest daily mortality rate occurred among adult midges handled the most (group A) (Fig. 2). Anesthetizing and blood feeding presumably contributed to this higher mortality rate. Adult midges handled the least (i.e., female adults emerging from pupae in the containers) (group C), experienced the lowest daily mortality rate (Fig. 2). The combined mean cumulative mortality rates for the two colonies were 52 ± 4% and 73 ± 3% at 20°C and 26°C, respectively. The ANOVA results revealed significant differences in mortality rates from day to day (Table 1). A significant handling by day interaction (Table 1) was also revealed; the significant differences in mortality rates due to the amount of handling were not consistent, but changed from day to day (Fig. 2). There were no significant differences noted between the four density levels of adult midges per container ($F = 0.296$; $df = 3, 173$; $P > 0.05$).

DISCUSSION

The mortality rate in adult *C. variipennis* (or other vector species) during the extrinsic incubation period for virus transmission is an im-

Table 1. Analysis of variance for the effects of environmental factors on mortality rates of adult *Culicoides variipennis*.

Source	*df*	*F*	Probability
Temperature	1	14.710	0.0001
Colony	1	0.006	0.9400 ns
Handling	2	12.440	0.0001
Day	14	2.286	0.0080
Handling × day	28	2.664	0.0001
Interactions (other)	56	—	ns
Error	437	—	—

ns = not significant.

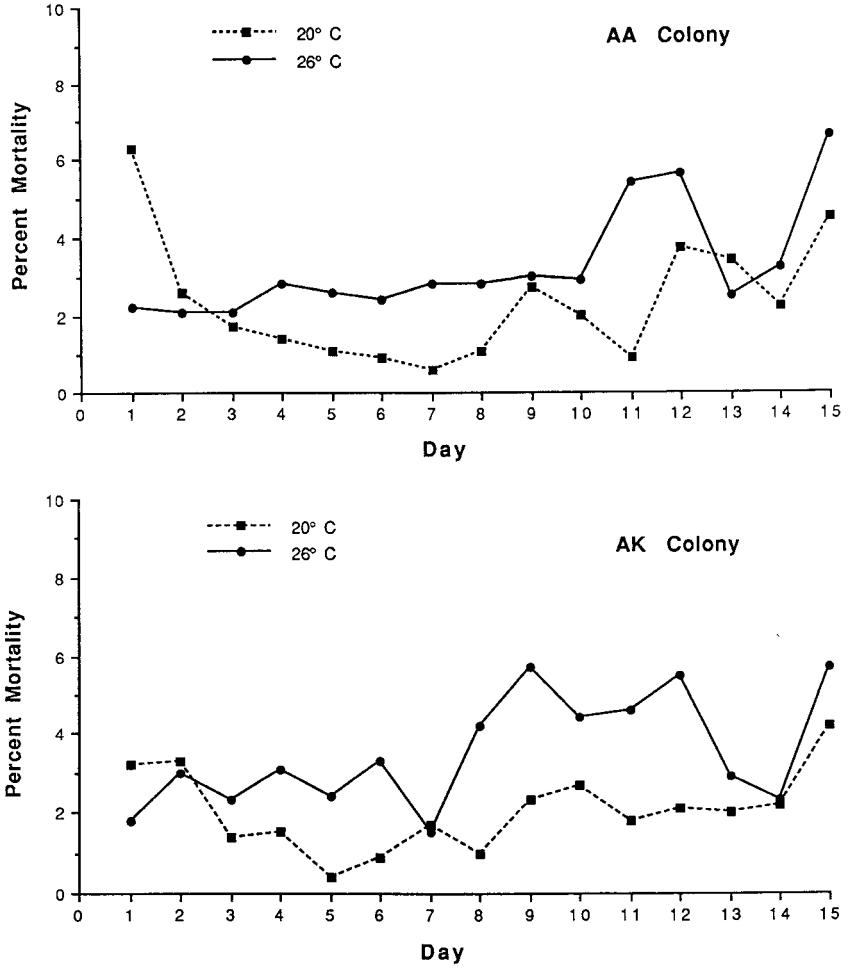

Fig. 1. Effects of two constant temperatures on daily mortality rates of adult *Culicoides variipennis* from two laboratory colonies. Points represent the average percent (n = 3 replicates) of daily mortality.

portant factor in conducting vector competence studies with disease agents. Poor survival of infected biting midges during this incubation period will limit the number of *C. variipennis* for subsequent feeding and, therefore, affect the dynamics of virus transmission.

Although mortality records from field or laboratory studies are relatively few for species of adult *Culicoides*, an increase in mortality at higher temperatures was expected. Similar observations have been reported for a variety of insect species; the cause of this relationship is complex and is attributed to a variety of factors (Sohal 1976). One significant factor is the influence of temperature on the insect's metabolic activity. In general, the metabolic rate tends to be inversely related to longevity (Sohal 1986). Even though the influence of constant temperatures on mortality rates of adult *C. variipennis* was studied under controlled laboratory conditions, temperature is not uniform throughout the environment of the insect's natural habitat.

The effect of increased handling on mortality rates of adult *C. variipennis* is of interest to investigators using this biting midge to study vector competence. The data presented here clearly show that the number of surviving insects will be enhanced if handling during routine maintenance procedures is minimized. It was expected that the effect of handling might be similar to the effect of density or overcrowding since each increases the chance of physical injury to adult *C. variipennis*. However, our results did not support this hypothesis using the density levels as previously described. Significantly different daily mortality rates were not observed

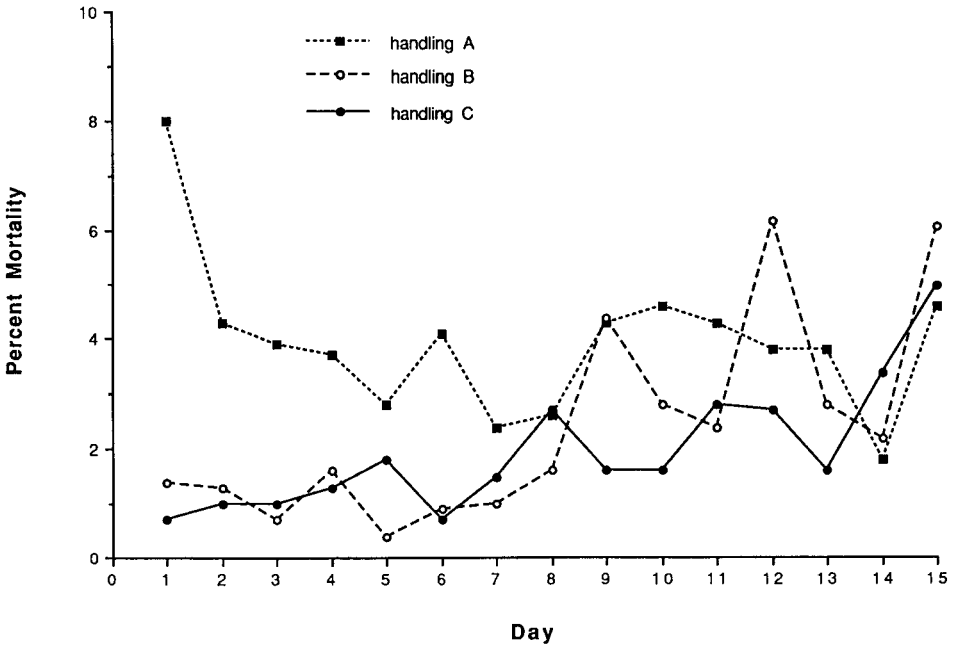

Fig. 2. Effects of three different handling techniques on the combined daily mortality rates of adult *Culicoides variipennis* from two laboratory colonies. Points represent the average percent (n = 6 replicates) of daily mortality. A = anesthetizing, blood feeding, anesthetizing, chilling, sorting; B = anesthetizing, chilling, sorting; C = none.

among the varying density levels of adult *C. variipennis* in the size of container routinely used in our laboratory.

The vector competence of natural populations of any vector species (i.e., *C. variipennis*) is dependent on a variety of extrinsic and intrinsic factors. The use of simple models to predict the vector competence of geographic populations is fraught with difficulties. For example, although high densities of a vector population are usually viewed as increasing the risk of disease, there actually may be fewer insects transmitting disease agents due to poor survival during the extrinsic incubation period at high temperatures.

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