ARTICLES

COLD TOLERANCE IN ADULT FEMALE *CULEX TARSALIS* (COQUILLETT)[1]

A. W. ANDERSON AND R. F. HARWOOD

The winter biology of *Culex tarsalis* (Coquillett) at northern latitudes is still not clearly understood. Because of the importance of this mosquito as the principal vector of western equine encephalitis and its possible role as the overwintering mechanisms of the virus, the winter biology must be clearly determined. The purpose of this investigation was to study the environmental factors of photoperiod, temperature, and humidity which affect this mosquito's successful hibernation.

Hibernation sites of this mosquito have received a great deal of attention but comprehensive studies of the actual environmental conditions necessary for successful hibernation are few in number. In western Nebraska, *C. tarsalis* was collected in food storage cellars (Keener, 1952) with no information on survival. Temperatures within these cellars varied from 0.56° to 10.6° C. Blackmore and Winn (1956) reported *C. tarsalis* hibernating in abandoned mines in Colorado, with temperatures from 12.2° to 13.3° C. and the relative humidity between 20 percent and 80 percent. In northern Utah, Dow *et al.* (1956) carried out overwintering studies of *C. tarsalis* in two abandoned mines with temperatures varying from 4.44° to 10° C. and the relative humidity from 67 percent to 82 percent. The authors stated that as the temperature in the mine increased, the mosquito seemed to be "forced" out of the mine. They further stated that if the energy source stored in the body must last throughout the winter, *C. tarsalis* would survive better in natural shelters where it would be inactivated by the cold.

C. tarsalis was reported as hibernating in talus slopes in Grant County, Washington (Rush, *et al.*, 1958). In a nearby man-made rockpile temperatures were lower than those previously reported in abandoned mines, 2.22° to 8.33° C., and the relative humidity fluctuated between 37 percent and 100 percent.

Trent (1960) carried out a study on the overwintering of *C. tarsalis* in rock slides in Utah. Temperatures varied from −1.67° to 3.33° C. The mosquitoes occupied approximately the same level in the rocks at any one time, suggesting selection of a preferred temperature and migration up or down within the talus slope as temperatures change.

Mail and McHugh (1961) captured hibernating *C. tarsalis* and *C. pipiens* from abandoned mines and subjected them to constant temperatures and various humidities in the laboratory. Maximum survival for both species was attained with high humidity at or a little below freezing.

METHODS. Two distinct strains of *Culex tarsalis* were tested and compared for cold tolerance in this study. The California strain, as noted by Bellamy and Kardos (1958), was started in 1952 from wild stock collected near Bakersfield, California (ca. 35° N). Our culture was started from egg rafts from this colony in 1960, and was not selected for autogeny. In addition to tests made with this unmodified culture, an attempt was made to develop a more cold resistant strain.

A northern strain was started from larvae, pupae, and adults collected during the summer of 1963 near Othello, Washington, Adams County (47° N). Hibernat-

[1] Partial financial support by Grant AI-2253 from the National Institutes of Health, U. S. Public Health Service is gratefully acknowledged. College of Agriculture, Washington State University, Scientific Paper 2703, Project 1434.

ing individuals were collected in February of 1964 from talus slopes in this same area and were also subjected to cold tests.

The larvae were reared in incubators with a light source connected to a time clock to automatically maintain the desired photoperiod. Long and short daily photoperiods of 16 and 8 hours respectively were used because Harwood and Halfhill (1964) found these day lengths affect fatbody development of *C. tarsalis* maximally.

Because of difficulties in obtaining accurate humidity measurements in the field and differences in humidities reported by various authors, three widely different humidities were evaluated. Arbitrarily, 20 percent, 50 percent, and 90 percent relative humidities were selected. Humidity-regulating solutions of potassium hydroxide (Buxton and Mellanby, 1934) were used because they absorbed excess carbon dioxide in the environment and avoided possible toxic effects from the evaporation of other humidity-regulating solutions.

The humidity chambers consisted of quart mason jars. These were convenient because they took little space in the cold cabinets. The mosquitoes were contained in a 2-inch square hinged plastic insect display cage which had perforations on all sides to permit free passage of air.

In the early experiments, simulated hibernation temperatures of —4° C. and 3° C. were used. Survival in these tests was poor, and temperatures were changed to 0° and —4° C. Further studies indicated that survival was better at —2° C. and the remainder of the tests were run at 0° and —2° C.

Mortality exceeded 50 percent in less than two weeks in the earlier tests, suggesting that some exposure to cold was necessary before the mosquitoes were subjected to the test temperatures.

A fairly complex cold preconditioning was then investigated (Fig. 1). The mosquitoes were kept on an 8-hour daily photoperiod (photophase) continuously at 22° C. for approximately 12 days after median adult emergence. Then during the dark period (scotophase) temperature was lowered to 10° C. for approximately 4 days. After this the scotophase temperature was lowered one degree C. daily to 0° C., and at the same time the photophase temperature was lowered at intervals to 10° C. The mosquitoes were next kept at 10° C. during the photophase and at 0° C. during the scotophase for approximately 15 days. Cotton pads soaked in 10 percent sucrose solution were always available throughout the preconditioning period.

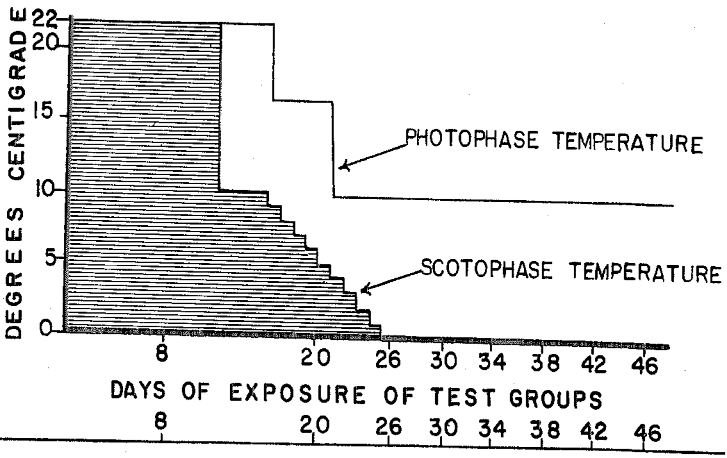
Early experiments indicated a poor cold tolerance in the California strain. Attempts were made to select a cold tolerant strain from the California mosquitoes by subjecting the second larval instars and adults to 0° C. for 48 hours. The intention was to destroy 80 percent to 90 percent of the mosquitoes and to obtain eggs from those which survived this cold challenge. This procedure was carried out through 8 generations, and from the fifth generation on, the step-wise preconditioning previously described was used.

Larvae and pupae of *C. tarsalis* were collected in Adams County August 1, August 14, September 10, and October 5, 1963, to determine if natural seasonal variation in cold tolerance occurred. Pupae of these mosquitoes were placed in 8-hour photoperiod at 22° C. through 7 to 10 days of adult life, and were then kept 10 more days at 10° C. before hibernation tests. Adults were provided with 10 percent sucrose solution throughout this preconditioning period. To relate natural hibernation to temperatures during larval development, average maximum and minimum temperatures for July, August, and September were obtained from the U. S. Weather Bureau at Othello, Washington.

RESULTS. Preliminary tests on the relationship between photoperiod and subsequent cold survival showed that short photoperiod invariably provided greater survival. This was the case whether maximum survival of paired comparisons was short or relatively long. The short photoperiod groups always survived two to three times as long. Consequently all subsequent tests were conducted at short photoperiod prior to cold survival exposures.

In the first tests conducted with the California-derived strain of *C. tarsalis,* survival at 0° and —4° C. was poor. The small hibernation chambers made it necessary to use 10 or less mosquitoes for each test. Cabinet space prevented replication in most cases. The LT_{50} (time in days for 50 percent mortality to occur during exposure to specific temperatures and humidities within the hibernation chamber) was 7 days or less at all humidities. The step-wise preconditioning procedure was used with two later groups and an increase in cold tolerance was evident. The LT_{50} at the higher humidities (50–90) ranged between 15 and 25 days at 0° C. but at —4° C. it was between 8 and 15 days. At 20 percent relative humidity survival was consistently poor, and this moisture level was dropped from further experiments. The remainder of the experiments were conducted at 0° C. and —2° C.

The cold-selected California strain was tested through 8 generations (Fig. 2).



TIME IN DAYS TO LT_{50}

0C	90% r.h.	16	26	18	18	19	16	11	21
	50% r.h.	10	11	15	12	10	12	11	11
−2C	90% r.h.	12	10	11	12	15	14	16	18
	50% r.h.	8	9	12	7	8	12	10	15

FIG. 1.—Cold survival of the F_6 generation of cold-selected California *C. tarsalis* during complex cold conditioning, 8 hour daily photoperiod.

Only a slight increase in cold tolerance was evident and this increase was not rapid. Survival was better at 90 percent relative humidity than at 50 percent and slightly better at 0° C. than at —2° C.

Preliminary tests were run with the colonized northern strain. The mosquitoes were preconditioned at 10° C. for 24 hours before exposure to test temperatures. In all cases survival was much better than that recorded with the California strain under the same conditions. At 0° C. and 90 percent relative humidity the LT_{50} was 38 days. In one experiment with 100 percent relative humidity and at 0° C. the LT_{50} was 73 days. No tests utilizing the step-wise preconditioning procedures were carried out.

Cold tolerance and survival in the wild northern group was better than for any

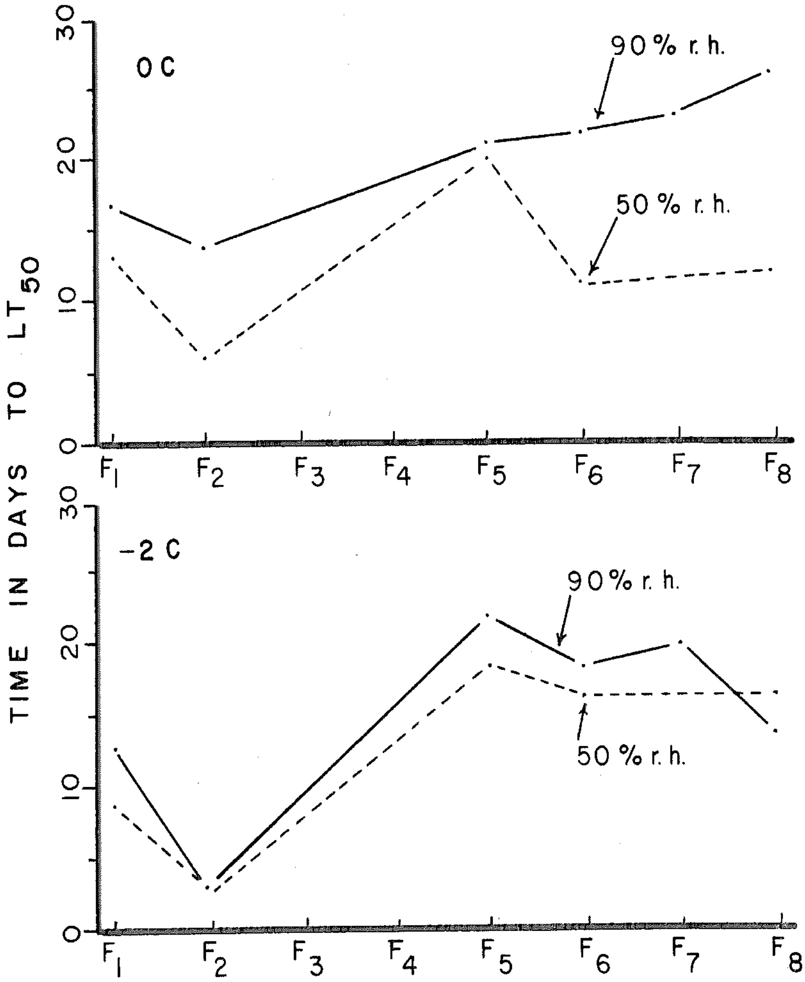


Fig. 2.—Cold tolerance of succeeding generations of the cold-selected California strain of *C. tarsalis*.

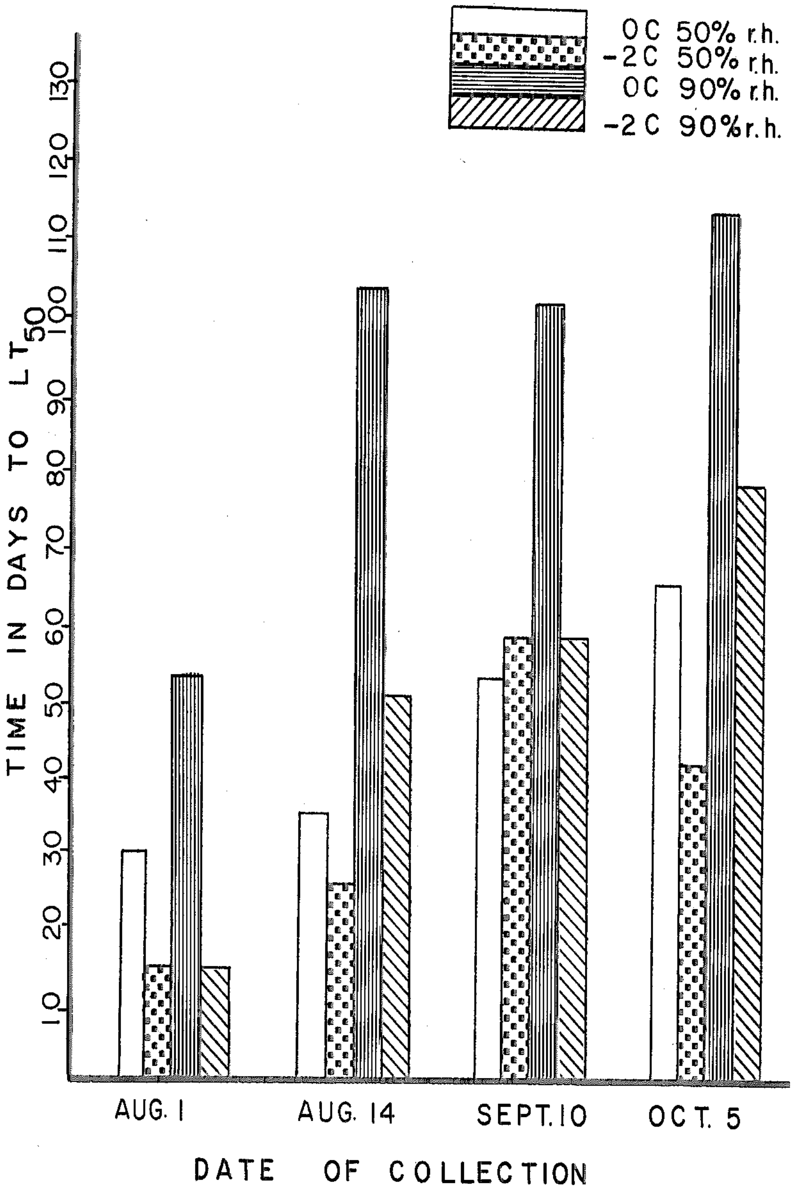


FIG. 3.—Cold tolerance of field collected eastern Washington *C. tarsalis*.

other group tested. An increase in cold tolerance was evident with each group collected as the late summer season progressed, and this increase was greatest at 0° C. and 90 percent relative humidity (Fig. 3).

Hibernating *C. tarsalis* were collected from talus slopes in Adams County on February 4, 1963. These were maintained up to one day at 0 C. enroute to the laboratory. The mosquitoes were kept at 90 percent relative humidity and were tested at —2°, 0°, 5°, and 10° C. The 50 percent survival times at these temperatures were 66, 54, 54 and 20 days respectively. Obviously a detailed comparison with other laboratory tests is not possible; however, prior winter survival at low temperatures added to that observed in the laboratory makes it clear that this group is at least as hardy as any of the others tested. The maximum survival rate carried 50 percent of the population into early April, the time when most adults would normally be out of hibernation in nature (Harwood, 1962).

DISCUSSION AND CONCLUSIONS. Climatic conditions during the winter inactive period vary greatly throughout the range of *Culex tarsalis*. In California it is 2 to 3 months; in central Washington about 6 months, but here temperatures below zero degrees Fahrenheit occur for only short periods. In North Dakota, such temperatures occur commonly during the hibernation period and may persist for 2 weeks or more, with the winter period at times exceeding 6 months.

In the fall, the mosquito must build up sufficient energy reserves to survive through the inactive period. It seems likely that if the hibernation site were too warm, high metabolic rates would cause these energy reserves to be more rapidly utilized, and survival over long periods would be jeopardized. Short photoperiod is necessary for maximum energy reserve accumulation (Harwood and Halfhill, 1964).

Relative humidity in an overwintering site is also a factor in successful survival during the inactive period, since the mosquito cannot replace water by nectar feeding or sucking of plant juice. Under laboratory hibernation conditions *C. tarsalis* reached maximum survival in 90 percent relative humidity. Survival at 20 percent r.h. was poor at any temperature and survival at 50 percent r.h. was generally poorer than at the higher humidity. Apparently the optimum humidity range for the overwintering mosquito is not extremely narrow, though it must be near saturation. Survival was maximal in these tests at a relative humidity of 90 percent, but survival in a saturated environment was often nearly as good.

While maximum survival occurred at 0° C. the mosquitoes survived better at —2° C. than at temperatures above zero. This suggests a fairly narrow optimum temperature range above and below which survival through a long hibernation period is reduced. Field data on a population of *C. tarsalis* hibernating in talus slopes indicated that in nature the mosquito will move up or down in the habitat as outside temperatures increase or decrease (Trent, 1960). This movement indicates that the mosquito actively orients itself during hibernation to remain within an area of optimum temperature.

Cold tolerance in *C. tarsalis* increases with the progression of late summer into early fall in eastern Washington. This increase was evident in the second group tested which was collected on August 14, 1963. Maximum survival of 114 days was observed in the group collected on October 5, 1963. This final collection consisted primarily of last stage larvae; therefore, rearing was completed under laboratory conditions.

Data from the U. S. Weather Bureau revealed that the average maximum temperature for the month of September, 1963, was 85.2° F., and the average minimum temperature was 52.9° F. Average maxima and minima for July and August of the same year, however, were nearly the same: 85° and 55° for July and 89.4° and 59° F. for August. Mean temperature ranges did not vary excessively from July through September, but the cold tolerance of wild mosquitoes increased when the adults in all groups were subjected to the same experimental conditions. This suggests that shorter photoperiod conditions in the fall increase cold tolerance. All the experiments with laboratory cultures of *C. tarsalis* substantiate this observation, particularly those subjected to short photoperiods both as larvae and adults rather than as adults only.

In California, blood feeding and ovarian development are reduced in the fall when temperatures are higher than in January or February when blood feeding increases (Bellamy and Reeves, 1963). These authors speculated that this is a diapause in response to shortened day length. In Colorado, Bennington *et al.* (1958) reported a drop in blood engorgement about August 1 and an increase in fat body development, beginning August 15. Laboratory studies to date indicate that shorter photoperiod conditions characteristic of late summer and early fall induce an increase in fat body size, as well as other physiological changes in fatty acid saturation of *C. tarsalis* which tend to prepare them for hibernation (Harwood and Halfhill, 1964, Harwood and Takata, 1965).

The wild hibernating mosquitoes collected in talus slopes on February 4, 1964 survived best at —2° C. Because of small numbers collected, only six mosquitoes were used in each test. All were alive after 46 days at 0° C. and 59 days at —2° C. Then mortality at 0° C. was fairly rapid, and at —2° C. it was somewhat slower. After 82 days (April 26), one mosquito was alive at 0° C., and two were still alive —2° C. At the lower temperature it appears that the mosquitoes did not utilize their energy reserves as fast as at warmer temperatures, and therefore survived longer. Better survival of these field-collected mosquitoes at the lower temperature differs from tests conducted with laboratory strains derived from the same latitude. This suggests additional factors in nature (possibly dietary) which increase the cold-hardiness of *C. tarsalis.*

Literature Cited

BELLAMY, R. E., and KARDOS, E. H. 1958. A strain of *Culex tarsalis* Coq. reproducing without blood meals. Mosq. News 18:132–4.

BELLAMY, R. E., and REEVES, W. C. 1963. The winter biology of *Culex tarsalis* (Diptera: Culicidae) in Kern County, California. Ann. Entomol. Soc. Amer. 56:314–23.

BENNINGTON, E. E., SOOTER, C. A., and BAER, H. 1958. The diapause in adult female *Culex tarsalis* Coquillett (Diptera: Culicidae). Mosq. News 18:299–304.

BLACKMORE, J. S., and WINN, J. R. 1956. A winter isolation of western equine encephalitis virus from hibernating *Culex tarsalis* Coquillett. Proc. Soc. Exptl. Biol. Med. 91:146–8.

BUXTON, P. A., and MELLANBY, K. 1934. The measurement and control of humidity. Bull. Ent. Res. 25:171–6.

DOW, R. P., MAIL, G. A., and RICHARDS, C. W. 1956. Observations on the overwintering of *Culex tarsalis* in northern Utah. Proc. Ninth Ann. Mtg. Utah Mosq. Abatement Assoc., pp. 12–15.

HARWOOD, R. F. 1962. Trapping overwintering adults of the mosquitoes *Culex tarsalis* and *Anopheles freeborni.* Mosq. News 22:26–31.

HARWOOD, R. F., and HALFHILL, E. 1964. The effect of photoperiod on fat body and ovarian development of *Culex tarsalis.* Ann. Entomol. Soc. Amer. 57:596–600.

HARWOOD, R. F., and TAKATA, N. 1965. Effect of photoperiod and temperature on fatty acid composition of the mosquito *Culex tarsalis.* Jour. Ins. Physiol. 11:711–16.

KEENER, G. G., JR. 1952. Observations on overwintering of *Culex tarsalis* Coquillett (Diptera: Culicidae) in western Nebraska. Mosq. News 12:205–9.

MAIL, G. A., and MCHUGH, R. A. 1961. Relation of temperature and humidity to winter survival of *Culex pipiens* and *Culex tarsalis.* Mosq. News 21:252–4.

RUSH, W. A., BRENNAN, J. M., and EKLUND, C. M. 1958. A natural hibernation site of the mosquito *Culex tarsalis* Coquillett in the Columbia River Basin, Washington. Mosq. News 18:288–93.

TRENT, D. W. 1960. Observations on the hibernation of *Culex tarsalis* Coquillett in Utah Valley, Utah. Unpublished Master's Thesis, Brigham Young University, Provo, Utah.