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Seasonal Abundance and Survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a Southern California Dairy, with Reference to Potential Bluetongue Virus Transmission and Persistence

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J. Med. Entomol. 37(5): 675–688 (2000)

ABSTRACT Seasonal abundance and survivorship of *Culicoides sonorensis* Wirth & Jones were examined at a dairy in southern California from January 1995 to December 1997. Insects were collected one to two times per week using five CDC-type suction traps (without light) baited with CO_2 at a constant release rate of 1,000 ml/min. Female and male abundance was greatest during late summer and early fall and was directly correlated with mean monthly air temperature. Parity of females was lowest during late summer and early fall. The gonotrophic cycle was estimated to require 3–4 d during hot summer months and up to 14 d during cool winter months. Estimated extrinsic incubation of bluetongue virus (BLU) was 9–10 d during August and September. The estimated daily survival ranged from <60% in the summer to >95% in the winter, resulting in an expectation of life of only 2–3 d in summer and >10 d in winter. The probability of females surviving the extrinsic incubation period for BLU virus, and the expectation of infective life were both lowest during late summer and early fall. During 1997, midge abundance during late summer was not high enough to overcome very low survivorship, and the absolute number of females expected to survive the extrinsic incubation period was relatively low. However, in 1995 and 1996, very high midge abundance compensated for low survivorship during summer and the number of females expected to survive the extrinsic incubation period was relatively high. Although abundance was generally very low during the cool winter and spring, host-seeking females were captured throughout the year, and their winter survival was high. Overwintering of BLU virus by continued transmission of the virus by active midges appears possible.

KEY WORDS *Culicoides sonorensis*, seasonal abundance, survivorship, bluetongue virus, vectorial capacity

VECTORIAL CAPACITY IS a measure of the likelihood of pathogen transmission by a vector population to a susceptible host population (Garrett-Jones 1964). Biting intensity (bites/host/time) and survivorship are two of the principal parameters that determine vectorial capacity. These two parameters are largely dependent on environmental conditions and would be expected to vary seasonally. Knowledge of the timing and magnitude of these seasonal variations is critical to understanding the epidemiology of any vector-borne disease. Other parameters of vectorial capacity, such as vector competence, might also be subject to environmental effects (Hardy et al. 1983, Wellby et al. 1996, Mellor et al. 1998). However, competence is also influenced substantially by genetic factors (Jones and Foster 1974, Tabachnick 1991) and therefore might vary somewhat less over time (Birley et al. 1984).

The biting midge *Culicoides sonorensis* Wirth & Jones is the primary vector of bluetongue (BLU) virus to ruminants in the United States (Foster et al. 1963, Luedke et al. 1967, Holbrook et al. 2000). This species is distributed widely throughout the southern and western United States, and generally is found in close association with polluted waters and livestock (Holbrook et al. 2000).

The Chino Basin Dairy preserve in southern California is ideal for the study of seasonal variation in vectorial capacity of *C. sonorensis.* Nearly 100% of all the *Culicoides* spp. collected in CO_2-baited traps in this region are *C. sonorensis* (unpublished data) and host-seeking females have been collected throughout the year (Mullens 1985). Additionally, serological surveys of slaughter cattle in southern California have demonstrated a high level of BLU virus transmission to ruminants (Metcalf et al. 1981), and cattle exposure to BLU virus may exceed 50% (Osburn et al. 1981, Stott et al. 1985).

Biting intensity is determined by the abundance of vectors and hosts, environmental factors, and the interval between blood meals. Because *C. sonorensis* displays gonotrophic concordance in the laboratory (one blood meal per gonotrophic cycle) (Hunt 1994), the length of the gonotrophic cycle is assumed to reflect the length of time between bloodmeals. Biting intensity can be estimated from the abundance of host-seeking *C. sonorensis* females captured in CO_2-baited traps, because the relationship of collections in these traps to actual numbers of females feeding on hosts has been determined (Mullens and Gerry 1998).

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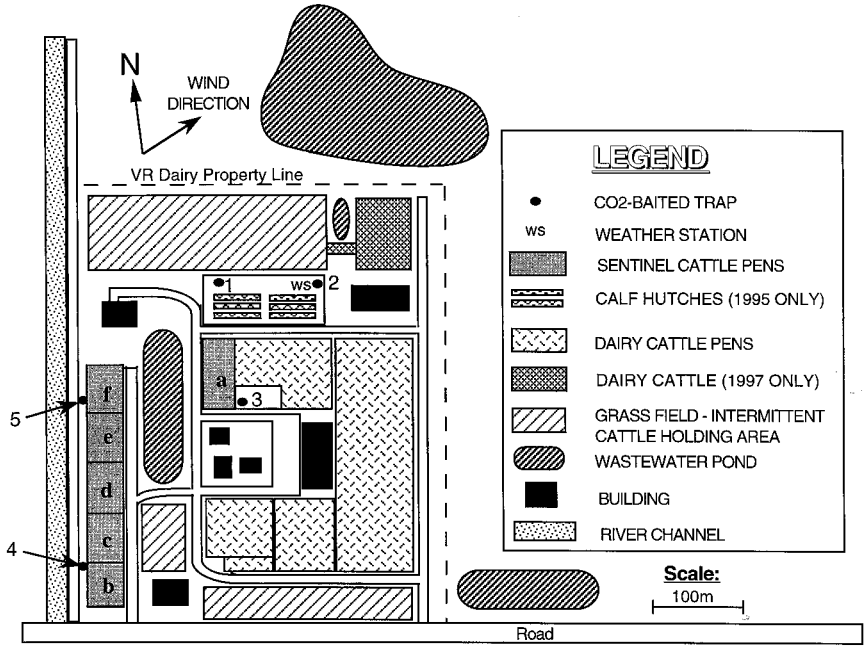

Fig. 1. Dairy site layout. The dairy was centrally located in the Chino Dairy Preserve, San Bernardino County, CA. Adjacent wastewater ponds belonged to neighboring dairies. Calf hutches near sites 1 and 2 were removed during 1996 and this area remained open for the remainder of the study. The cattle pen in the northeast corner of the dairy was constructed in 1996, but did not hold cows until 1997. Winds were generally from the southwest, except for the few days each year during Santa Ana wind conditions when the winds blew from the east.

Relatively minor changes in survivorship of a vector can theoretically greatly affect the transmission of a disease agent, such as BLU virus, that requires a period (the extrinsic incubation period) after infection of a vector before transmission is possible (Garrett-Jones 1964; Dye 1990, 1992). The expectation of infective life is therefore determined by both survivorship and the pathogen extrinsic incubation period (MacDonald 1952). Davidson (1954) proposed that survivorship can be estimated from parity rates (number parous females/number total females) if the length of the gonotrophic cycle is known.

The primary objective of the current study was to determine seasonal changes in abundance and parity of host-seeking female *C. sonorensis* over a 3-yr period at a site with a history of bluetongue virus activity. Seasonal variability in abundance and parity has been shown for some *Culicoides* spp. (e.g., Walker 1977a, 1977b; Birley and Boorman 1982; Akey and Barnard 1983; Braverman et al. 1985; Kramer et al. 1985; Linhares and Anderson 1989; Venter et al. 1997). Multiyear studies are critical to understand how environmental conditions may alter these important parameters of vectorial capacity.

Materials and Methods

Study Area. Studies were conducted at a privately owned dairy centrally located in the Chino Basin dairy preserve of southern California (western Riverside and San Bernardino counties). This ≈75-km^2 area has >250,000 milking cows. Most dairies in this region have wastewater ponds, which are close to cattle, that provide larval habitat for *C. sonorensis* (Mullens 1989).

Several wastewater ponds on and near the cooperating dairy provided development sites for *C. sonorensis* (Fig. 1). A centrally located wastewater pond generally held water from April through August. A small wastewater pond was constructed on the northeastern portion of the dairy during the final year of the study, but was steep-sided and did not support much larval development. Large wastewater ponds that held water throughout the entire 3-yr study were located 75 m northeast and 50 m southeast of the dairy. Wastewater ponds were not sampled regularly for *C. sonorensis*, but large numbers of larvae and pupae were collected from the pond southeast of the dairy on a number of occasions in summer and fall throughout the study period.

A battery-operated hygrothermograph was placed inside a covered weather station on site, and temperature/humidity charts were replaced weekly. Because of hygrothermograph malfunctions, 65 d during the 3-yr study did not have on-site temperature and humidity data. These days were predominantly in the first year of the study (40 of 65 d) and all were from December through March. On-site temperatures for these days were estimated using the daily maximum and minimum temperatures recorded at the University of California at Riverside, 32 km east of the dairy

(NOAA, Climatological Data, California, 1994–1997, Asheville, NC). Daily maximum temperatures recorded for 5-d periods before and after each block of missing data were highly correlated ($R^2 = 0.88$) between this site and the field site.

Weighted degree-day (DD) values were computed for the coldest month (January) during each year. Sellers and Mellor (1993) determined a "weighted degree-day" value for the coldest month of the year above which *Culicoides* spp. were associated with endemic transmission of BLU virus. This value is computed by assigning a score of 6 to days with maximum temperatures of 18–29°C, and a score of 1–5 to temperatures of 13–17°C, respectively, summing the score for each day of the month, and dividing the total by the number of days in the month.

Insect Collections. CDC-type miniature suction traps (John W. Hock, Gainesville, FL), with the light removed, were placed at five locations (trap sites 1–5) around the dairy (Fig. 1). Traps were suspended 0.7 m above ground and 10 cm below a gray 3.8-liter paint can. Traps were baited with CO_2 gas released from a tank with a two-stage regulator at a rate of 1,000 ml/min, measured using a flow meter. This rate is roughly equivalent to the amount of CO_2 produced by a nearly grown Holstein heifer (Roberts 1972), and attracts primarily host-seeking insects (Holbrook 1985, Anderson and Linhares 1989). Insects were collected into an organdy catch bag attached to a 237-ml cardboard container supplied with a vial of 10% sucrose with a cotton wick.

Traps were deployed 2 h before sunset and removed 2 h after sunrise. Host-seeking by female *C. sonorensis* occurs mainly within this period (Nelson and Bellamy 1971, Barnard and Jones 1980a, Akey and Barnard 1983, Linhares and Anderson 1990, Mullens 1995). This trapping schedule also accommodates minor seasonal fluctuations in diel patterns of host-seeking or parity (Barnard and Jones 1980a, Akey and Barnard 1983, Mullens and Rutz 1984, Anderson and Linhares 1989, Mullens 1995).

Traps were operated from January 1995 through December 1997; one night per week during winter and spring (December–June) and two nights per week during summer and fall (July–November) when midges were more abundant. Summer and fall were presumed to be the seasons of greatest BLU virus transmission (Osburn et al. 1981, Loomis et al. 1985, Stott et al. 1985, Uhaa et al. 1990, Gibbs and Greiner 1994). Trapping occasionally was postponed for 1 or 2 d during rainy periods, but otherwise was conducted regardless of weather conditions.

Captured insects were returned to the laboratory, anesthetized with CO_2, and placed on a chill table to be sorted and counted by sex and parity status. Parity was determined by the presence of pigment deposited in the abdominal cuticle (Dyce 1969, Akey and Potter 1979) and by changes in the pigmentation pattern of abdominal tergites (Potter and Akey 1978).

Determination of Survivorship. Mean daily survivorship was estimated from mean parity rates of captured females (Davidson 1954). Therefore, p is estimated by $P^{1/u}$ where p is the daily probability of survival, P is the parity rate (number parous females/number total females), and u is the length of the gonotrophic cycle. To account for variable parity within a cohort, monthly parity (calculated from the sum of samples) was used to estimate survivorship from June–November, whereas survivorship during winter and spring was calculated using parity over 3-mo periods (December–February and March–May, respectively).

The gonotrophic cycle reflects the length of time required for host location, blood feeding, egg maturation, and oviposition. It was assumed that the majority of the gonotrophic cycle was composed of oogenesis, because of the abundance of cattle and the close proximity of dairy wastewater ponds for oviposition (i.e., host availability and oviposition sites were not limiting). The relationship between temperature and time to oviposition after blood feeding has been investigated (from 13 to 34°C) and a regression equation ($y = -1.98 + 0.07217x + 2516.65x^{-2}$) has been determined such that the time to oviposition (y) can be calculated for any temperature (x) (Mullens and Holbrook 1991).

Expectation of Infective Life. Vertical transmission of BLU virus by *C. sonorensis* has not been demonstrated (Jones and Foster 1971, Nunamaker et al. 1990). Therefore, to transmit BLU virus, females must ingest virus, survive the viral extrinsic incubation period, and take a subsequent blood meal. The probability of surviving the extrinsic incubation period can be estimated as p^n, where p = the daily probability of survival and n = the extrinsic incubation period of the disease agent (MacDonald 1952). The expectation of life after surviving the extrinsic incubation period (the expectation of infective life) is then given by $p^n/-\log_e p$ (MacDonald 1952).

The rate of virogenesis of BLU virus influences the length of the extrinsic incubation period and varies with environmental temperature (Mullens et al. 1995). Data previously reported for BLU virus (Foster et al. 1963; Luedke et al. 1967; Foster and Jones 1973, 1979; Chandler et al. 1985; Mullens et al. 1995; unpublished data) were plotted (Fig. 2) and a linear regression equation was determined ($y = -1.03x + 36.79$) ($R^2 = 0.83$) such that for a given temperature (x), the length of the extrinsic incubation period (y) could be estimated. Because of the lack of data at lower temperatures and the apparent absence of viral replication at ≤15°C (Mullens et al. 1995), the use of this equation was restricted to months when the mean monthly temperature was ≥18°C (June–October).

Statistics. Catches in CO_2-baited traps were transformed by $\log_{10}(n+1)$ and subjected to analysis of variance (ANOVA) using the GLM procedure in SAS System 6.12 with year, month, and trap site as main effects (SAS Institute 199). Means for significant main effects were separated using the Tukey honestly significant difference (HSD) test.

Parity rates for each trap catch were transformed by arcsine-square root and tested as above. Parity was analyzed by ANOVA only for the months of June

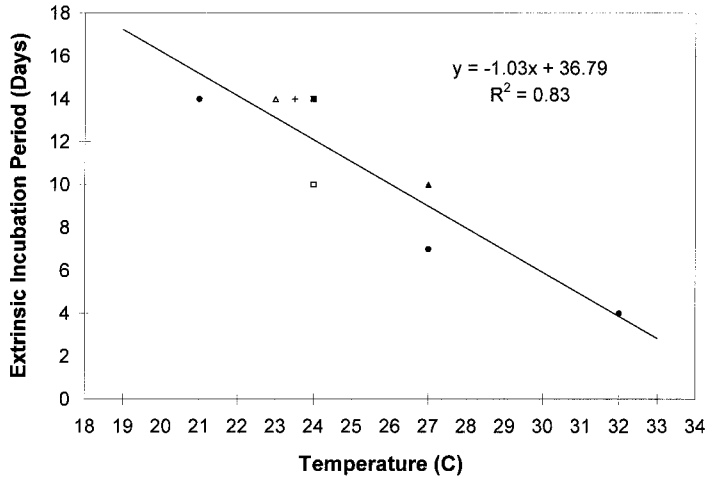

Fig. 2. Extrinsic incubation period of BLU virus plotted as a function of incubation temperature. Based on data from Mullens et al. (●, unpublished data, 1995); △ Foster and Jones 1979; + Foster and Jones 1973; ■ Luedke et al. 1967; □ Foster et al. 1963; ▲ Chandler et al. 1985.

through November when trap collections were ≥10 females. Means for significant main effects were separated using the Tukey HSD test. Mean parity levels (numbers parous and nulliparous) for winter (December–February), spring (March–May), and paired summer and fall months (June–July, August–September, and October–November), were subjected to chi-square analysis using Minitab (Minitab 1996) to examine year-round differences in parity.

Data for trap site five were not included in the above analyses due to a decrease in midge capture at this site following the addition of a nearby cattle pen (pen "f" in Fig. 1) on 13 September 1995. However, data from site five collected after this date were used to calculate the mean abundance and parity for combined sites 3–5. Mean abundance and parity were used to determine daily survivorship, expectation of infective life, and number of females expected to survive the BLU virus extrinsic incubation period for each combined site group (1 and 2 and 3–5).

Mean abundance and parity for the week before and the week after periods of very low humidity (periods covered at least one consecutive night and day) were calculated, and their difference was subjected to a sign test using Minitab (1996).

Results

Weather. Mean monthly maximum (MMmax), mean monthly, and mean monthly minimum (MMmin) temperatures were estimated using the daily maximum, mean, and minimum (respectively) temperature for each day of the month (Fig. 3). Whereas, mean daily temperatures occasionally exceeded 30°C in summer and early fall and dropped below 6°C during the winter and early spring, mean monthly temperatures just exceeded 26°C during summer and fall and usually remained above 12°C during the winter months. Absolute minimum temperatures of −0.5 to 1°C occurred each winter of the study and an absolute maximum temperature of 42°C occurred each summer and fall.

The MMmax temperature for the coldest month in each year was >16°C and daily maximum temperatures rarely fell below 13°C. The maximum number of consecutive days with mean daily temperature <13°C was 4 d in January 1997. During 1995 and 1996, MMmax temperatures increased from May through August, when temperatures peaked at 36–37°C. In 1997, the MMmax temperature was quite high during May (31°C), decreased in June, and then increased through September where it peaked at 34°C. There were more months in 1997 with MMmax temperatures above 25°C (8 mo) than in 1995 (6 mo) or 1996 (7 mo). However, the MMmax temperature of the hottest month was lower in 1997 than in 1995 or 1996. A weighted degree-day value (Sellers and Mellor 1993) for January was computed as 3.04, 4.08, and 3.90 for 1995, 1996, and 1997, respectively.

Monthly precipitation data were recorded at the University of California at Riverside (NOAA, Climatological Data, CA, 1994–1997, Asheville, NC) (Fig. 3). The 1994–1995 rainy season (November–May) had 47.83 cm of precipitation, well above the normal 23.1 cm (30-yr average). The 1995–1996 and 1996–1997 seasons were approximately average for this region (18.21 cm and 21.74 cm, respectively).

On eight occasions during October–November over the 3-yr study, the humidity remained very low for at least 24 h, reflecting high wind conditions ("Santa Ana Winds") that are common in the fall in southern California. Neither abundance nor parity differed significantly between the weeks before and the weeks after these periods of low humidity ($P = 0.73$ and $P = 0.29$, respectively).

Abundance. A total of 95,338 female and 8,964 male *C. sonorensis* was captured in the five CO_2-baited suction traps over the 3-yr study. The number of males

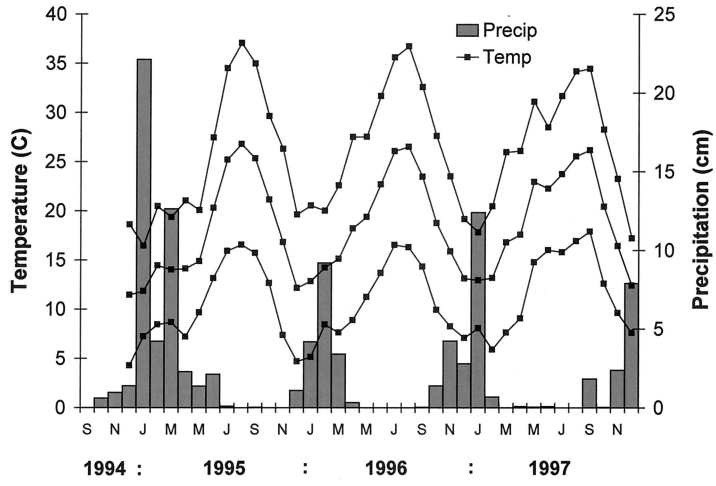

Fig. 3. Mean monthly maximum, mean, and minimum temperatures measured by on-site hygrothermograph, and total rainfall recorded by the National Oceanic and Atmospheric Administration at the University of California at Riverside for each month of the study.

captured was correlated with the number of females captured for each collection night ($r = 0.77$, df = 217, $P < 0.001$). Of the total females captured, 19,383 were parous (20.3%) and therefore potential carriers of BLU virus. The highest trap capture occurred on 8 August 1995 with 2,597 females and 152 males at site 1.

Abundance of females varied with year and month of capture ($F = 4.47$; df = 2, 842; $P < 0.05$ and $F = 151.35$; df = 11, 842; $P < 0.001$, respectively). Abundance peaks were relatively consistent in timing, but varied in amplitude for each of the 3 y (Fig. 4). Significantly fewer females were captured during 1996 than in either 1995 or 1997, which were not significantly different from each other. The largest numbers of both females and males were collected in late summer and early fall (August–September) with collections often ≥300 females per trap night, whereas in the colder winter and spring months, collections of ≤10 females per trap night were common. Although winter and spring abundance was low, host-seeking midges were captured throughout the year. Mean monthly abundance of females (sites 1–4 combined) was significantly correlated over time with mean monthly field temperature ($r = 0.76$, df = 34, $P < 0.001$).

Abundance of females also differed among trap sites ($F = 182.50$; df = 3, 842; $P < 0.001$), but the number of host-seeking females captured was significantly correlated among all trap sites over time ($0.548 < r < 0.774$, df = 163–214, $P < 0.001$) (Fig. 5). The majority of host-seeking females were captured at trap sites 1 and 2 ($n = 58{,}915$ or 61.8% and 21,389 or 22.4%, respectively). These trap sites were furthest from the cattle pens and nearest to the open pasture and large dairy pond northeast of the dairy. Substantially fewer females were captured at sites 3–5 ($n = 3{,}103$ or 3.3%, $n = 6{,}026$ or 6.3% and $n = 5{,}905$ or 6.2%, respectively), which were nearest to the cattle pens. Similarly, the majority of males were captured at sites 1 and 2 ($n = 5{,}023$ or 56% and $n = 2{,}938$ or 32.8%, respectively), with substantially fewer captured at sites 3–5 ($n = 324$ or 3.6%, $n = 408$ or 4.6% and $n = 271$ or 3%, respectively).

Survivorship. Parity of host-seeking females varied significantly by year and month (June–November) of capture ($F = 4.82$; df = 2, 429; $P < 0.01$ and $F = 6.86$; df = 5, 429; $P < 0.001$, respectively). Parity during June–November was lower for 1996 (24%) than for 1995 (30%) or 1997 (28%). Parity did not differ between winter and spring months or between late fall and winter months, but was significantly different for all other pairwise comparisons (Table 1). Parity during winter and spring was high (33.8% and 34%, respectively), as was parity during paired months in late fall (31%). Parity during paired months in summer and early fall was much lower (20.8% and 17.5%, respectively). Monthly parity was negatively correlated with mean monthly field temperature ($r = -0.66$, df = 34, $P < 0.001$) and mean monthly female abundance ($r = -0.50$, df = 34, $P < 0.005$).

Parity also differed by site of capture ($F = 22.96$; df = 3, 429; $P < 0.001$). The interaction of year and site effects was not significant ($F = 1.87$; df = 6, 372; $P = 0.084$). Trap sites 1 and 2 were furthest from the cattle pens, and average parity at these sites (23 and 22%, respectively) was lower than average parity at sites 3–5 (30, 36, and 30%, respectively), which were adjacent to the cattle pens (Fig. 6). Mean monthly parity for combined sites 1 and 2 was negatively correlated over time with mean monthly field temperature ($r = -0.68$, df = 34, $P < 0.001$), whereas parity for combined sites 3–5 was not correlated over time with mean monthly field temperature ($r = -0.166$, df = 34, $P = 0.33$). Parity at sites 3–5 was weakly correlated with parity at sites 1 and 2 ($r = 0.495$, df = 34, $P < 0.005$).

Gonotrophic cycle length varied from a low of 3–4 d during summer to a high of 14 d during winter (Fig. 7). Daily probability of survival was calculated sepa-

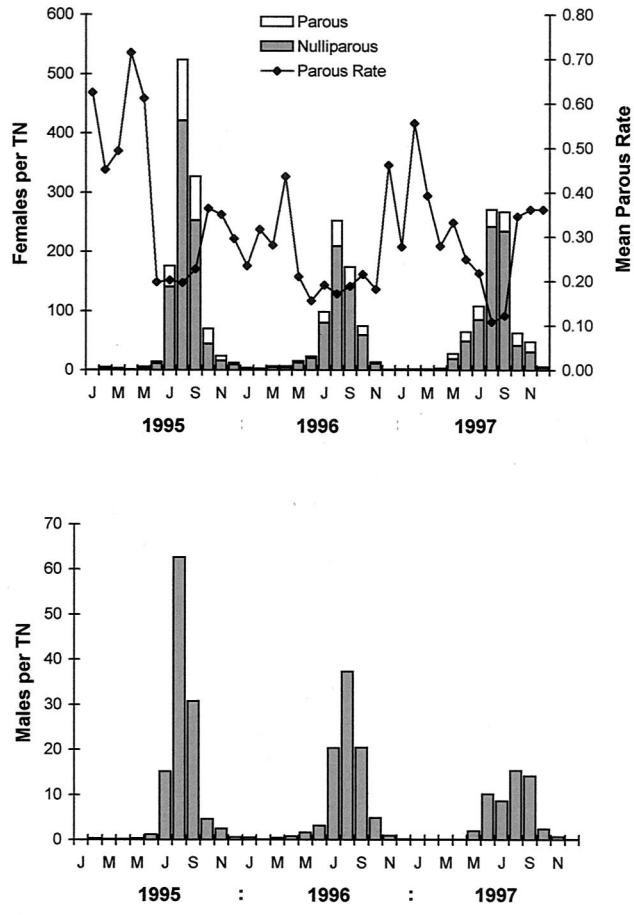

Fig. 4. Mean per trap night (TN) female (parous and nulliparous) and male *C. sonorensis* by month, and mean monthly parity of captured females at trap sites 1–4.

rately for sites 1 and 2 and sites 3–5 because of the significant differences in parity between these groups of traps. Daily probability of survival varied from 0.53 to 0.95 for trap sites 1 and 2 and from 0.62 to 0.97 for trap sites 3–5, and it was consistently higher from late fall through spring than during summer and early fall (Fig. 8). The expectation of life determined by daily survivorship was as short as 2 d during the summer and generally exceeded 10 d during the winter (20 d during 1995) (Fig. 8). The expectation of life decreased exponentially with increasing temperature, reaching a minimum of ≈2 d even at the highest temperatures (Fig. 9).

Expectation of Infective Life. The extrinsic incubation period for months where the mean monthly temperature was ≥18°C (June–October) varied from a low during August and September of ≈9–11 d to a high of 14–18 d in June and October (Fig. 7).

The probability of surviving the extrinsic incubation period was consistently higher when calculated for sites 3–5 (0.01–0.10) versus sites 1 and 2 (0.001–0.05) (Fig. 10). Because of the low probability of survival at both site groupings, the actual number of females expected to survive the extrinsic incubation period was very low, even when midge abundance was very high during summer.

During 1995 and 1996, the number of females expected to survive this period increased from June through August, with numbers decreasing thereafter. During 1997, the number of females expected to survive this period was relatively high during June and July, decreased during August and September, and peaked during October.

Although the expectation of infective life was <1 d for each month analyzed, it was consistently greater for sites 3–5 than for sites 1 and 2 (Fig. 11). At sites 1 and 2, the expectation of infective life was nearly zero for every month except October during all 3 yr. At sites 3–5, the expectation of infective life in 1995 and 1997 was relatively high in early summer, decreased during late summer and early fall, and increased again during October. In contrast, the expectation of infective life at sites 3–5 during 1996 was relatively low throughout the summer and increased during October.

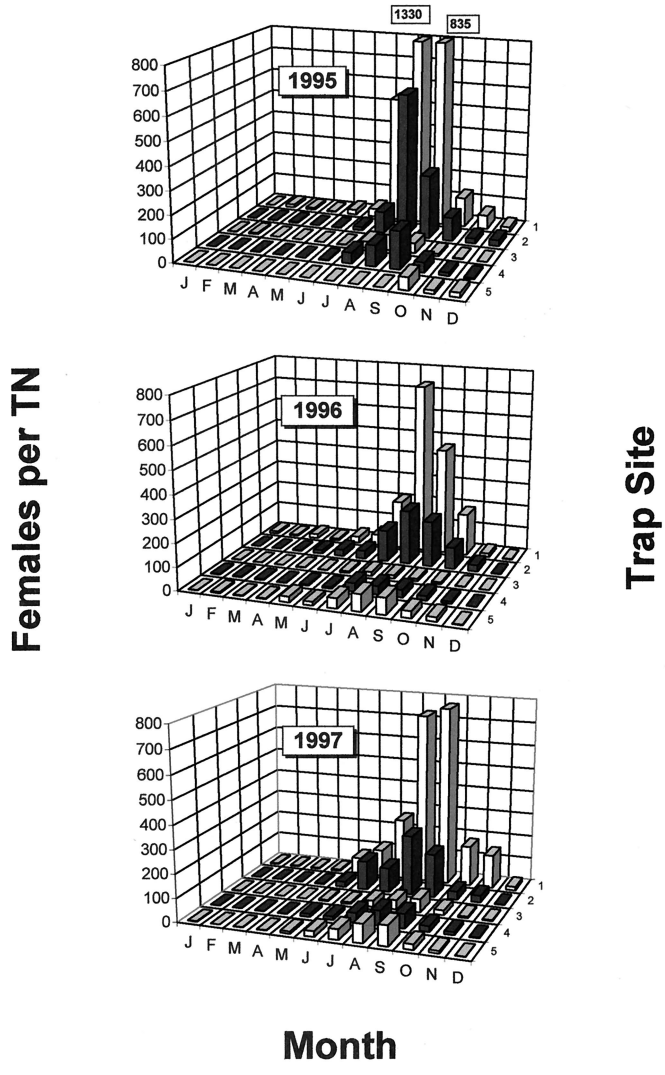

Fig. 5. Mean monthly capture of female *C. sonorensis* per trap night (TN) for each trap site (1995–1997). Collections at trap site 5 before October 1995 are not shown.

Discussion

Weather. Most dairies in the Chino Basin have wastewater ponds providing relatively stable, year-round development sites for *C. sonorensis.* Nevertheless, greater midge abundance in 1995 compared with 1996 and 1997 may have been caused by the much greater rainfall during 1995, which increased the number and size of suitable ephemeral breeding sources as wastewater ponds overflowed and flooded nearby pastures.

Temperatures affect *C. sonorensis* flight activity and thus influence trap capture. Using vehicle-mounted nets, Nelson and Bellamy (1971) rarely collected *C. sonorensis* at temperatures >32°C or <10°C. However,

Table 1. Total numbers of parous and nulliparous midges, and percent parity of *C. sonorensis* collected between 1995 and 1997

	Collection period				
	Dec.–Feb.	Mar.–May	June–July	Aug.–Sept.	Oct.–Nov.
Parous	202	411	2,964	11,079	3,094
nulliparous	396	798	11,291	52,294	6,894
% parous	33.8ab	34.0a	20.8c	17.5d	31.0b

Parity estimates followed by the same letter are not significantly different ($P > 0.05$) by pairwise chi-square comparison.

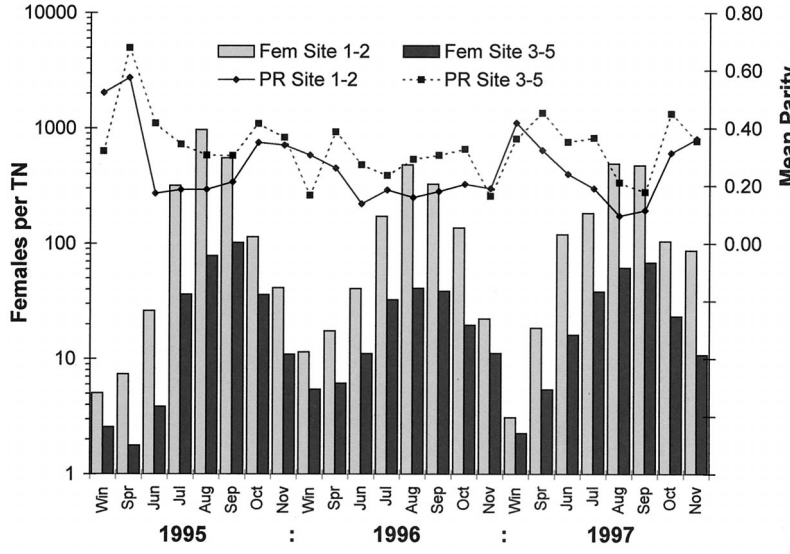

Fig. 6. Mean monthly capture of female *C. sonorensis* (n+1) per trap night (TN) and mean monthly parity for trap site groups 1 and 2 and 3–5. Winter (December–February) and spring (March–May) are combined because of low abundance during these periods.

others have collected *C. sonorensis* females in the field at temperatures as low as 7°C (Barnard and Jones 1980a, Linhares and Anderson 1990), and Mullens (1995) collected low numbers of females at a dairy near our study site when temperatures were between 5 and 7°C.

At our study site, daily maximum temperature was <10°C on only 2 d throughout the entire study (both in 1995 and both >7°C). Flight activity was therefore possible year-round at this site.

Abundance and Parity. Seasonal abundance trends were similar during each year of the study. Abundance rapidly increased during the early summer months, peaked in late August and early September, declined rapidly from October through December, and remained at low levels through April. Host-seeking nulliparous females were captured throughout the winter months, indicating that larval development and emergence continued during this period. Mullens and Lii (1987) found *C. sonorensis* larvae (second to fourth instars) in dairy ponds in the Chino Basin throughout the winter and spring. The correlation of abundance and mean monthly temperature indicated that adult midge abundance was influenced by larval development rates, which increased as a function of temperature (Mullens and Rutz 1983). Similar seasonal abun-

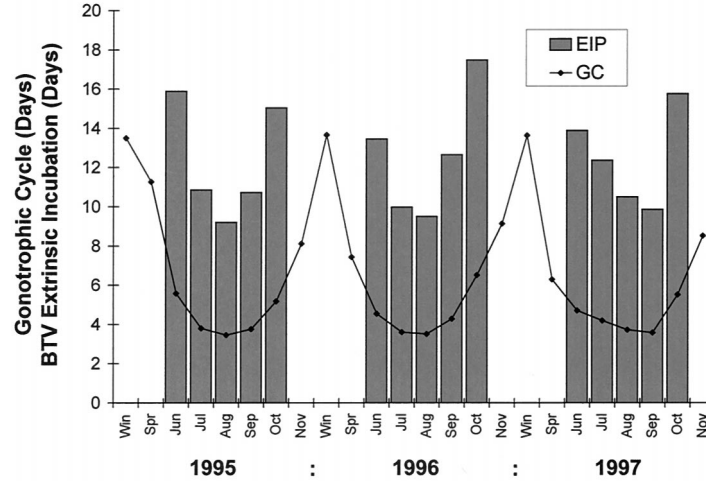

Fig. 7. Estimates of BLU virus extrinsic incubation period and gonotrophic cycle length for *C. sonorensis* based on mean monthly field temperature. Extrinsic incubation period is not estimated for November, winter or spring because of the lack of published data regarding BLU virogenesis at low temperatures. Mean temperature during these periods was <18°C.

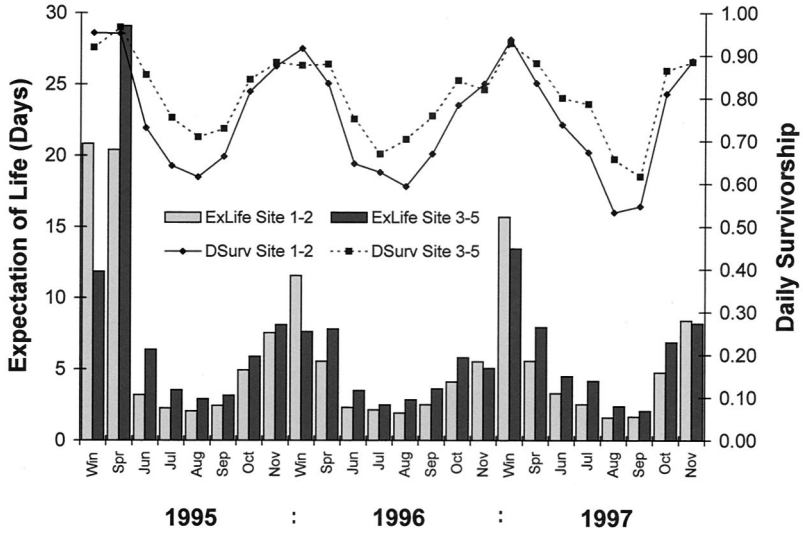

Fig. 8. Estimates of daily survivorship and expectation of life for female *C. sonorensis* based on mean monthly parity and gonotrophic cycle length at trap site groups 1 and 2 and 3–5.

dance patterns have been shown previously for *C. sonorensis* adults (Loomis et al. 1985) and larvae (Mullens and Lii 1987) in southern California, as well as adult *Culicoides imicola* and *C. oxystoma* Kieffer in Israel (Braverman et al. 1985), adult *C. imicola* in South Africa (Nevill 1971, Venter et al. 1997), and adult *C. imicola* in Spain (Ortega et al. 1999). The rapid decrease in abundance from late September through October was likely a reflection of decreasing temperature prolonging larval development, decreasing adult emergence, and decreasing feeding frequency as the length of the gonotrophic cycle was increased. Developmental sites (dairy wastewater ponds) were still plentiful throughout this period.

Parity was low over an extended period during summer and early fall, and remained low when adult abundance began to decline following the summer peak. This extended period of low parity indicated that adult survival was low during this period. Parity increased rapidly in late fall to early winter and remained at relatively high levels over an extended period until early summer, indicating that adult survival was high during this period. In contrast, *Culicoides* spp. in South Africa increased in both parity and abundance toward the end of summer (Venter et al. 1997).

Site Differences. Midge abundance and parity differed substantially between trap sites 1 and 2 and sites 3–5. Greater midge abundance at trap sites 1 and 2 may have been the result of the large permanent wastewater pond 75 m northeast of the dairy (≈350 m from sites 1 and 2) (Fig. 1). Midges host-seeking upwind (winds were generally from the southwest) would not encounter animals before reaching these trap sites, except on unusual days when the grass field to the

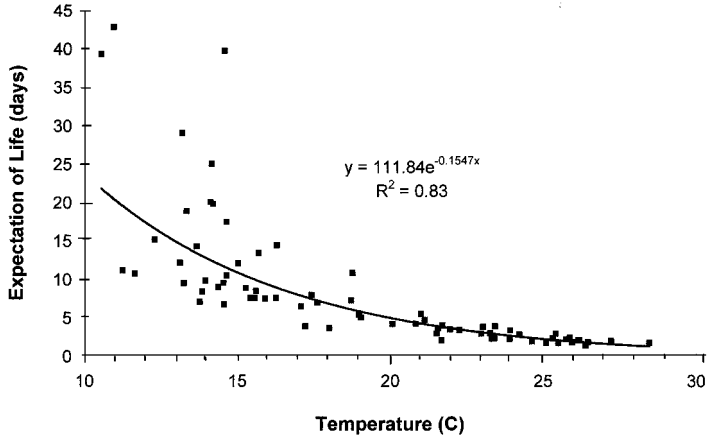

Fig. 9. Expectation of life for female *C. sonorensis* plotted as a function of field temperature. Expectation of life was determined for each 2-wk period of the study using mean parity.

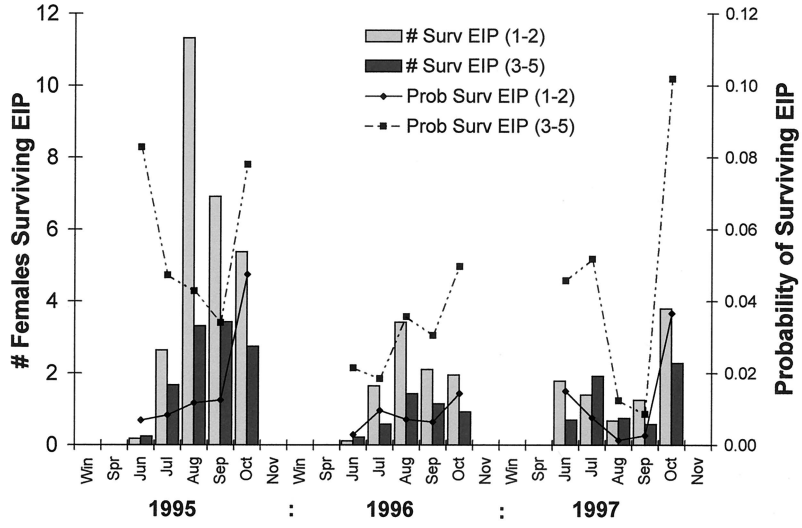

Fig. 10. Estimates of the probability of surviving the extrinsic incubation period and the number of females expected to survive this period for each trap site group (1 and 2 and 3–5). Values for November, winter and spring were not estimated because of lack of BLU virus extrinsic incubation data at low temperatures.

north was in use to pasture animals. Trap sites 3–5 were adjacent to cattle pens, and would compete for host-seeking female midges with nearby dairy cattle. Mullens and Gerry (1998) collected approximately seven times as many midges from bait calves relative to suction traps baited with calf-equivalent amounts of CO_2. Sites 4 and 5 were directly upwind of cattle pens, and host-seeking midges generally would have to fly past cattle to reach these traps. Site 3 was the only truly internal site at the dairy, and had the lowest overall abundance during each month.

Zimmerman and Turner (1984) found that the percentage of nulliparous females increased with distance from a breeding source, presumably because of upwind dispersal of nullipars before initiation of host-seeking behaviors and an immediate return to host-seeking by recently ovipositing parous midges. Mullens (1985) and Work et al. (1991) showed that CO_2-baited suction traps very near a development site were biased in favor of parous *C. sonorensis.*

Parity was lower at trap sites 1 and 2 relative to sites 3–5 from March through October. Although sites 1 and 2 were closer to the pond northeast of the dairy relative to sites 3–5, they may have been far enough from the pond (≈350 m) that nulliparous females dispersing upwind were responsive to CO_2. Females blood

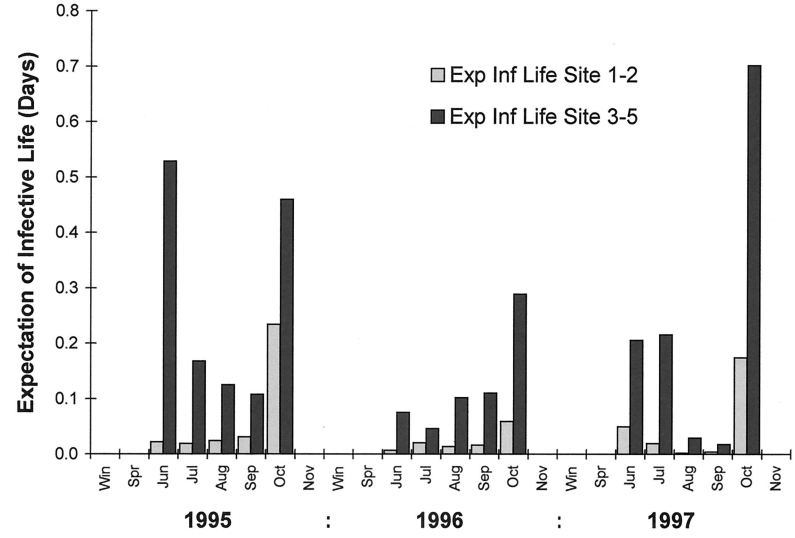

Fig. 11. Expectation of infective life estimated for each trap site group (1 and 2 and 3–5). Values for November, winter and spring were not estimated because of lack of BLU virus extrinsic incubation data at low temperatures.

feeding on dairy cattle may have oviposited at acceptable larval development sites near the dairy pens (such as the temporary central wastewater pond), thereby maintaining high parity near sites 3–5 relative to sites 1 and 2. In contrast, there was a rapid decrease in parity at sites 3–5 after the month of October during each year, and parity during the cold months of the year tended to be lower at sites 3–5 relative to sites 1 and 2. This rapid decrease in parity at sites 3–5 may be the result of the loss of oviposition sites (e.g., the central wastewater pond) near the cattle pens. The only oviposition sites available during these cold months may have been the large permanent dairy ponds at the periphery of the dairy. After oviposition, parous females would be expected to respond immediately to CO_2 and feed on cattle near the peripheral dairy ponds (and far from sites 3–5). In contrast, nulliparous females would disperse some distance before host-seeking and would be more likely to be captured at the trap sites furthest from the permanent ponds.

Survivorship. Dye (1992) suggested that for a seasonal vector population lacking a stable age distribution, the parous rate will correctly estimate the survival rate if calculated from the sum of samples taken throughout the season of activity. To account for cohort variation in the parous rate caused by emergence, samples were summed over periods that corresponded to the generation interval for *C. sonorensis.* Mullens and Lii (1987) determined this to be 3.0–4.5 wk in summer or 6–8 wk in winter, and Linhares and Anderson (1989) estimated a generation interval for *C. sonorensis* in northern California to be 2.5–4.0 wk during the summer. The higher negative correlation between the parous rate and temperature ($r = -0.66$) than between the parous rate and abundance ($r = -0.50$) indicates that low summer survival was more a result of increasing temperature than increasing adult density, although these were difficult to separate. For example, small summer midges (Mullens 1987) may not survive as well as large winter midges. This relationship has been documented for several species of mosquitoes (Haramis 1983, Nasci 1986, Day et al. 1990, Kitthawee et al. 1990).

The gonotrophic cycle for *C. sonorensis* was calculated to require 3–4 d during August and September. Work et al. (1991), using time series analysis, estimated the gonotrophic cycle length of *C. sonorensis* in California during August to be 3 d. *C. imicola* in Israel had an estimated gonotrophic cycle of 3–4.5 d during August and September (Braverman et al. 1985) and 5–6 d during May and June (Birley et al. 1984). The similarity of these estimates to our own supports using the time required to complete oogenesis and oviposition as an approximation for the length of the gonotrophic cycle provided hosts and oviposition sites are nearby and abundant.

Estimated daily probability of survival was lower at trap sites 1 and 2 than at sites 3–5 for every period except winter and the month of November. Survival at trap sites 1 and 2 was lowest during August of each year (53–62%) and highest during winter (92–95%). At sites 3–5, the period of lowest survivorship varied among years (71% in August 1995, 67% in July 1996, and 62% in September 1997). The period of highest survivorship also varied for each year; 97% in spring 1995, 88% in winter and spring 1996, and 93% in winter 1997. Our estimates of survivorship during the summer months were close to those of Work et al. (1991), who found daily survivorship of *C. sonorensis* during August to be 0.62 in northern California and between 0.57 and 0.81 in southern California.

As field temperatures increased, the expectation of life decreased exponentially reaching a minimum of ≈2 d. This pattern of survival may significantly increase the transmission of BLU virus at high summer temperatures if the extrinsic incubation period of BLU virus continues to shorten in a linear fashion at these high temperatures.

Extrinsic Incubation Period. *C. sonorensis* can transmit BLU virus to animals or embryonating chicken eggs after a 10- to 14-d extrinsic incubation period at 21–24°C (Foster et al. 1963, Luedke et al. 1967, Foster and Jones 1973). BLU virus transmission to embryonating chicken eggs was estimated to require minimum extrinsic incubation periods of 4, 7, and 14 d at 32, 27, and 21°C, respectively (B.A.M., unpublished data). These times corresponded to maximum virus levels detected by enzyme-linked immunosorbent assay for insects held at these temperatures (Mullens et al. 1995).

No BLU virus replication was observed over 22 d at 15°C, whereas higher temperatures were associated with an increased rate of virogenesis. Foster and Jones (1979) demonstrated that *C. sonorensis* orally infected with BLU virus developed a maximum viral titer after 14 d incubation at 23°C, which was stable through day 35 after infection. This is likely a conservative estimate of the extrinsic incubation period, because midges may be capable of viral transmission before the maximum viral titer is reached. Chandler et al. (1985) used a direct immunoflourescent technique to demonstrate BLU virus presence in head squash preparations of orally infected *C. sonorensis* after 10 d incubation at 27°C. For *C. sonorensis* orally infected with a related Orbivirus (African horse sickness virus), virogenesis occurred above 15°C and maximum virus titer was reached at 9 and 23 d after infection at 25 and 20°C, respectively (Wellby et al. 1996, Mellor et al. 1998).

Field temperatures often varied by 15°C or more each day, and midges may have spent a part of each day at temperatures below the apparent 15°C threshold for viral replication or above the maximum temperature used in laboratory BLU virus studies (32°C). Midge resting sites are unknown, but we assume that they rest in locations that would protect them from temperature extremes. Therefore, we have used mean monthly temperatures as appropriate to estimate the extrinsic incubation period.

Expectation of Infective Life. For every month that the extrinsic incubation period was estimated, it was over twice as long as the length of the gonotrophic cycle. Therefore, a female midge would be capable of transmitting BLU virus to a susceptible host only on the third and subsequent feedings after an infective

blood meal. Of 282 parous midges collected near our field site during 1982 and 1983 (Mullens 1985), ovarian dissections revealed 206 unipars, 60 bipars, 14 tripars, and two 5-parous midges. With summer survivorship at our study site near 0.70 per day (trap sites 3–5), and a gonotrophic cycle length of 3.5 d, we would expect 1 in 500 midges (or 1 in 143 uniparous midges) to survive five gonotrophic cycles. This estimate closely matches the ovarian dissection results listed above (Mullens 1985).

Estimated survivorship was greater at trap sites 3–5 than at sites 1 and 2. The probability of a female midge surviving the BLU virus extrinsic incubation period was therefore also greater at sites 3–5. However, the absolute number of females estimated to survive the extrinsic incubation period of BLU virus was greater at trap sites 1 and 2 because of the greater midge abundance at these sites. The pattern of females expected to survive the extrinsic incubation period of BLU virus during 1995 and 1996 was similar to the pattern of seasonal abundance. However, during 1997 the pattern appeared to be more heavily affected by survivorship.

The expectation of infective life varied substantially between the two trap site groups in our study, reflecting the sensitivity of the survivorship calculation to changes in parity. Using parity at trap sites 3–5 as the most epidemiologically relevant estimate of midge survival, the expectation of infective life, although low, was substantially higher than if we had combined all five traps. For all months calculated (June–October), the expectation of infective life was generally lowest during August and September and highest during October.

Viral Persistence and Overwintering. Environmental conditions during each year of our study exceeded those outlined by Sellers and Mellor (1993) for overwintering of BLU virus through continued transmission by active adult *Culicoides:* (1) the MMmax temperature of the coldest month was ≥12.5°C, (2) >45% of the daily maximum temperatures were ≥13°C, (3) weighted degree-day values for the coldest month were >1.35 (3.04, 4.08, and 3.90 for 1995, 1996, and 1997, respectively), and (4) fewer than 40 d per year and <10 consecutive days had maximum temperatures <13°C.

Although BLU virus has, to our knowledge, not yet been recovered from winter-collected insects, continued transmission of BLU virus by active insects may be an important maintenance mechanism in regions with mild winters. In Australia, overwintering of BLU virus apparently occurs in northern populations of *C. brevitarsis* Kieffer that are active year-round, with subsequent BLU virus transmission in the south after midge range expansion during the warm months of the year (Bishop et al. 1995, 1996). In South Africa, Venter et al. (1997) collected *Culicoides* year round in areas free of winter frost, and Nevill (1971) isolated BLU virus by testing enormous numbers of *Culicoides* captured immediately after winter, indicating that low-level virus transmission had occurred during the winter months. Winter persistence of BLU virus, or the closely related African horse sickness virus, in active *Culicoides* also has been suggested in Israel (Braverman et al. 1985), Spain (Rawlings and Mellor 1994), and Morocco (Baylis et al. 1997) where midges have been collected year-round. Southern Australia, South Africa, Israel, and southern Spain have temperature regimes similar to southern California (Sellers and Mellor 1993) and might be expected to have similar viral maintenance mechanisms during the winter months. In contrast, *C. sonorensis* in northeastern Colorado and perhaps central California overwinter as larvae (Nelson and Scrivani 1972, Barnard and Jones 1980b), and adult midges have not been observed during December–February in Colorado (Barnard and Jones 1980b) or midwinter (January and early February) in central California (Nelson and Scrivani 1972).

The possibility of BLU virus persistence in adult midges active during the winter months in southern California merits further investigation. Cattle do not serve as long-term reservoirs of BLU virus, and therefore are not a vital link in long-term virus maintenance (MacLachlan et al. 1990). Intensive winter collections of *C. sonorensis* in southern California may reveal persistence of BLU virus in active midges. Low-level transmission of BLU virus may be interrupted by applying control techniques (e.g., insecticide sprays) in areas where BLU virus persists throughout the winter months. Breaking the transmission cycle at this weak link may be an effective means of preventing bluetongue during the subsequent transmission season.

Acknowledgments

We are grateful for the tireless assistance of the following people in our laboratory: R. Velten, T. Chin, C. Szijj, and K. Luhring. We are thankful for the insightful reviews of E. T. Schmidtmann (Arthropod-Borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, WY), and two reviewers at the University of California, Riverside (M. S. Mulla and W. E. Walton) on an earlier version of the manuscript. We appreciate the willingness of the Van Ryn Dairy in allowing us to conduct these studies. The study was funded by USDA-NRICGP Grant No. 94-37204-1092 to B.A.M.

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Received for publication 19 October 1999; accepted 10 April 2000.