OBSERVATIONS ON SOME *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) LARVAL HABITATS IN AREAS OF BLUETONGUE VIRUS OUTBREAKS IN CALIFORNIA[1]

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ABSTRACT. *Culicoides variipennis* was the major species identified from rearing larvae and pupae collected from 36 of 98 sites examined in 10 counties of California. Most sites discovered were on or within close proximity to ranches citing previous or active bluetongue virus (BTV) cases. The sites are classified into habitat categories with a table of their frequency of occurrence and geographic distribution. Dairy waste-water lagoons are discussed in terms of their role as permanent, productive gnat habitats.

The importance of *Culicoides variipennis* (Coquillett) as the primary vector of bluetongue virus (BTV) in the United States has recently received increased attention. As a consequence of this disease, sheep may experience high mortality, loss of breeding season, abortion and deformed lambs (Erasmus 1975), while cattle may produce malformed or still-born offspring (Luedke et al. 1970, 1977). These facts, coupled with the vector's diversity of developmental sites, i.e. fresh-, salt-, and alkaline water soil habitats (Jones 1961), make this species a very realistic threat to the cattle and sheep industries in California.

MATERIALS AND METHODS

During the periods from September through November 1978, and from March through December 1979, routine surveys were conducted on potential *C. variipennis* larval sites in 4 areas of California. The selection of these areas (Sonoma County—northwest, Butte and Stanislaus Counties—Central Valley, and Imperial County—southwest cultivated desert) was based on prior unpublished veterinary reports of clinical BTV in sheep and from livestock blood samples reported BTV positive by diagnostic laboratories of the State Bureau of Animal Health and the School of Veterinary Medicine, University of California. Opportune surveys were made in 1979 to find *C. variipennis* larval sites in other counties because of clinical BTV outbreaks in sheep reported by regulatory and practicing veterinarians to the authors during the course of study (Fig. 1).

Sites were identified by collections of larvae and pupae in samples of substrate from the soil-water interface of suspected habitats. A 23.4 cc sample (7.5 × 6.5 × 0.5 cm) was removed with a trowel and placed in shallow water in a white circular pan. The water was gently swirled to help concentrate the debris and allow the larvae to become visible as they swam from the substrate to the perimeter of the pan. Once larvae were detected, samples were collected and placed in plastic bags for laboratory counting, rearing and identification of adults.

Field samples taken from polluted water sources were retained in the laboratory in 90 × 90 × 38 cm redwood bins that were lined with plastic and half-filled with soil. Fresh cow manure and water were added to a central depression in the soil to provide a suitable substrate for the larvae. Bin covers were fitted with white organdy to prevent escape of adults. Adults resting on the screened cover were collected daily with a modified hand vacuum and placed in cages for later identification.

When larvae were discovered at non-polluted

Fig. 1. California counties surveyed for *Culicoides variipennis* habitats: 1978, 1979.

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sites, they were placed in 21 × 11.5 × 5 cm white enameled pans in the laboratory with substrate taken from the habitat. Pupae were collected by flooding the pans and floating them to the surface. A circular piece of filter paper was placed atop a moist cotton pledget in the bottom of a 30 ml screw-top vial to hold pupae until adults emerged. Each vial cap was center cut and a small piece of organdy placed inside the cap to permit ventilation.

The maximum larval density at each site was based on a tentative index developed from field observations which estimated larval populations as: light < 10; moderate 11–100; dense 101–1000; very dense > 1000 larvae/trowel sample. With the exception of light densities of larvae which were almost always fourth instar, larval sites were characterized by the presence of various instars and pupae.

RESULTS

Of the 98 locations sampled, 36 *C. variipennis* larval sites were discovered and classified into 3 major and 12 specific habitats (Table 1).

A. AGRICULTURAL HABITATS

1. *Dairy waste-water lagoons.* Lagoon construction varied from concrete lined holding facilities (some with liquid-solid separators) to shallow excavations in unused pasture areas. Wash water from pre-milking operations, milk strippings, animal feed and waste were contained in these lagoons. In 9 of the 23 dairy waste-water lagoons sampled in 6 counties, larvae were most frequently found along the nonvegetated lagoon margins where the soil-water interface had a slope < 40° (beach-like, Fig. 2). These sites supported dense to very dense larval populations particularly from summer through fall months. Sites varied from small, discrete pockets of larvae to contiguous populations along one or more margins of the lagoons. Areas along the margins with thick vegetation appeared the least conducive larval sites having either very light or no larval populations present (Fig. 2).

2. *Dairy waste-water runoff.* In 2 counties, 5 dairies were found to empty their waste-water runoff either directly into a pasture or into drain ditches. This resulted in one dairy having approximately 2.0 ha of pasture covered by a depth of 5–30 cm of waste-water. Light to moderate densities were found around the perimeter of the pasture from spring to early summer months; islands of grass were created throughout the pasture from constant flooding and dense larval populations were recovered from the surrounding soil of each island in the fall months.

Runoff from the other 4 dairies was diverted into drain ditches bordering their pastures. Two of these ditches supported larval development. Dense larval populations at one dairy were found throughout summer to fall months of 1979 while at the other dairy only moderate populations were found at infrequent intervals throughout the same period. Larvae at both sites were found primarily along the soil-water interface where mats of algae and solid wastes had accumulated.

3. *Feedlots.* In 2 of 7 feedlots examined in one county, larval sites were established due to uncontrolled waste management. During in-

Table 1. Type and occurrence of *Culicoides variipennis* larval habitats in counties with bluetongue virus outbreaks, California, 1978–79.

	Number of sites sampled by county (% gnat positive)					
Habitat type	Butte/ Stanislaus	Mendocino/ Sonoma	Imperial/ Los Angeles	Kern/ Kings	Sacramento/ Yolo	Site totals
A. *Agriculture*						
1. Dairy waste-water lagoons	5(60)	4(25)	1(100)	1(100)	12(25)	23(39)
2. Dairy waste-water runoff	4(50)	1(100)				5(60)
3. Feedlots			7(29)			7(29)
4. Water trough overflow	2(50)				3(33)	5(40)
5. Irrigated pasture	3(33)			3(33)	1(100)	7(43)
6. Drainage canals			13(15)		4(25)	17(18)
B. *Urban/Industrial*						
1. Oxidation ponds		3(33)	8(63)			11(55)
2. Water containment ponds			5(40)			5(40)
3. Urban home runoff			1(100)			1(100)
4. Oil well runoff				3(33)		3(33)
C. *Natural*						
1. Rivers and creeks	4(25)	5(20)	4(25)			13(23)
2. Waterfowl refuge			1(100)			1(100)
Habitat totals	18(44)	13(31)	40(38)	7(43)	20(30)	98(38)

spections in 1978 at one feedlot, 4 shallow, irregular shaped pools were found in empty cattle pens as a result of both a broken underground water main and seepage from a cracked cement water trough. Inspections at the second feedlot in 1979 disclosed larval sites in numerous, intermittent, small pools of runoff water which formed along a shallow drain channel that led to a nearby river.

Both sites were characterized by a high concentration of animal wastes and varying amounts of algae. At the first feedlot dense larval populations were evenly distributed throughout most of the pools. Repair of the water main and trough in early 1979 resulted in elimination of these pools. No larval sites were found during the course of this study in feedlots which operated under full capacity and had good water management. Pool sites in the drain channel at the second feedlot were characterized by moderate larval populations with a less uniform distribution.

4. *Water trough overflow.* Two of five larval sites in 2 counties were found in accumulations of standing water around animal watering troughs. Two of these sites located on a dairy and a horse ranch were the result of broken float valves in the troughs. On the dairy, animal access was limited to one side of the trough with an undisturbed 1.5 m diameter larval site located on the opposite side.

Water trough overflow in the horse corral flowed down a 3 m long shallow channel and emptied into a slough. Larvae were found in the muddy hoofprints around the trough and in those bordering the channel to the slough. Moderate larval densities were repeatedly found throughout the summer and fall months at both sites.

5. *Irrigated pastures.* Larval sites were found in standing irrigation water in 3 of 7 pastures examined in 3 counties. Little or no vegetation was present in or around these sites. In the pastures examined, larvae were found in the hoofprints of resident swine, in muddy areas surrounding leaky irrigation valves in a dairy pasture, and at the margins of a shallow pond which formed from overflow crop irrigation in a sheep pasture. All 3 larval sites were exposed to animal traffic and were characterized by varying amounts of animal waste and algae.

Summer to fall month inspections showed light larval densities in the swine hoofprints and along the shallow pond margins but moderate densities persisted in the site around the irrigation valves. A light larval density was found

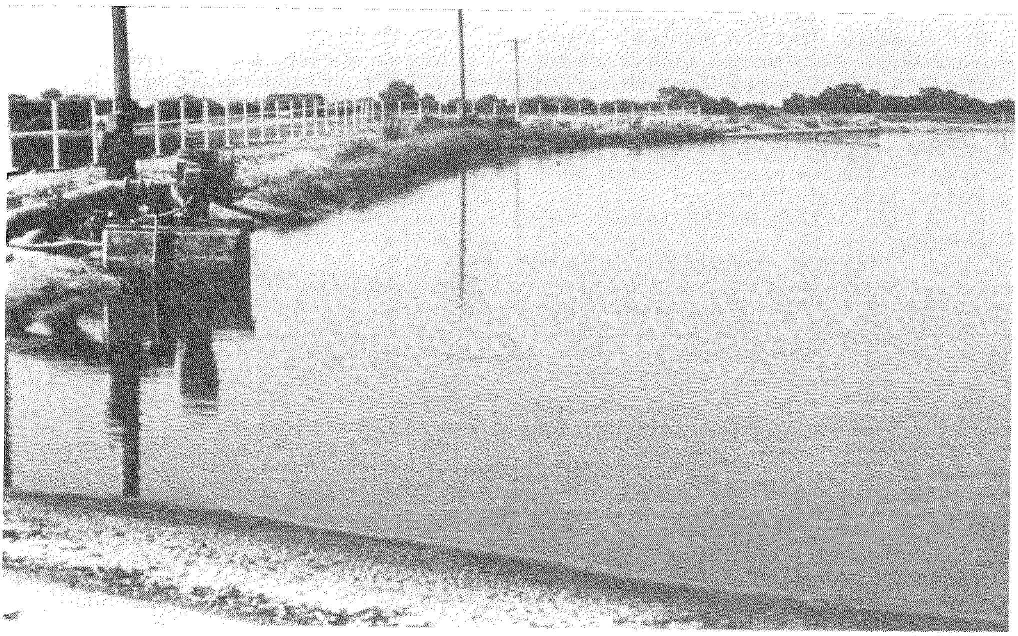

Fig. 2. Dairy lagoon with positive *Culicoides variipennis* beach-like margin (foreground) but negative along margin covered with vegetation (upper left).

scattered throughout the pond in the sheep pasture only during September 1979.

6. *Drainage canals.* Of the 17 drain canals examined in 2 counties, larvae were found in 3 which contained runoff from crop irrigation and in one which received both crop irrigation water and effluent from a nearby dairy. Low water levels between crop irrigations exposed wide sections of beach-like earthen margins and islands which consisted of soft, sandy mud and organic detritus. Moderate larval densities were repeatedly found during late summer and fall months in samples taken from the soil-water interface of these margins and islands which extended along the length (25 to 75 m) of these canals.

In contrast, the canal which received crop drainage and dairy effluent had a high-water level at all times and had no well-defined water-soil interface. Moderate densities of larvae were found in the soil surrounding clumps of vegetation which accumulated in certain areas along the margin of the 70 m long canal. Larvae found in these sites probably represented the remnants of established larval populations.

B. URBAN/INDUSTRIAL HABITATS

1. *Oxidation ponds.* In 3 counties, larvae were discovered during August and September 1979 in 6 of 11 sewage oxidation ponds which contained effluent from treated human sewage. Characteristics common to 5 of these larval sites were: flat, beach-like margins with vegetation absent, very little wave action, and soil composed of fine granules covered by a thin layer of silt (Fig. 3). The sixth site had steep, hard-packed margins with the leeward margin composed of large rocks to prevent erosion from wave action.

Light to moderate larval densities were found scattered along the margins of the first 5 ponds. In the sixth pond a light larval population was found on the leeward margin occupying empty chironomid pupal cases between the rocks.

2. *Water containment ponds.* Of the 5 sites examined in one county, larvae were found in numerous shallow excavations within a fallow pasture and in an evaporation pond on the premises of a drive-in theater. At the first site, wash water from cleaning livestock trailers drained directly into the pasture. Moderate larval densities were repeatedly present during the fall months in discrete areas along the soil-water interface of these sites.

Water at the second site originated from rest room sink drainage. This 6 m diameter pond had gently sloped beach-like margins free from any algae or debris. A moderate larval density was found almost continuously around the entire perimeter of the pond during the late summer and fall months of 1979.

3. *Urban home runoff.* One larval site discovered during fall months of 1978 consisted of a 7 × 1 m shallow, earthen ditch located in the yard of a private home. The ditch contained water from a garden hose, as well as animal waste from dogs, goats, ducks and horses. A light larval density was found at the soil-water interface which was covered by a thick mat of algae. This site was not a permanent source through the 1979 inspections due to irregular water supply governed by home owner use.

4. *Oil well runoff.* In Kern County, underground water from operational oil pumps produced a continuous flow of surface water which drained along one or more narrow, shallow channels. Channel margins consisted of loosely-packed sand covered with a thin layer of oil. Of the 3 sites examined, light larval densities were found during midsummer along the margins of one of these runoff channels. Wirth and Jones (1957) however, reported the rearing of large numbers of *C. variipennis* from similar sources while Nelson and Bellamy (1971) stated that waste water from oil fields provided larval sites for several *Culicoides* species.

C. NATURAL HABITATS

1. *Rivers and creeks.* Larvae were discovered in 3 of 13 sites in 3 counties: along the banks of a river at the high water level; from indentations along the banks of a fast-flowing creek; and in brackish water of a river estuary. The banks of the river contained a layer of rocks atop sandy soil while the banks of the creek and river estuary consisted of a fine soil free from rocks and debris. All 3 sites were devoid of vegetation.

Larval densities were light and widely scattered at each site throughout the sampling period in 1979. These immature gnat populations probably represented non-permanent and very marginal larval sites as a result of intermittent flooding.

2. *Waterfowl refuge.* Larval development was found in 4 newly constructed permanent ponds located between the Salton Sea and irrigated farmland in Imperial County. These brackish-water ponds, largely devoid of bank vegetation, created extensive habitat for migrating waterfowl as well as for the production of *C. variipennis*. Light gnat larval densities were present during late summer and fall months of 1979 in discrete groups along the gently sloped margins.

Fig. 3. Municipal sewage oxidation pond with positive *Culicoides variipennis* sites along nonvegetated beach-like margin.

DISCUSSION

Other *Culicoides* species besides *C. variipennis* were monitored from laboratory rearing of larval samples. According to Jones (1959), *C. crepuscularis* Malloch larvae are usually associated with *C. variipennis sonorensis,* at least in human sewage sites, but in such reduced numbers that they are often concealed by the massive populations of *C. variipennis.* An extremely light population of *C. crepuscularis* was found in a larval sample from only one dairy lagoon. Adults of *C. freeborni* Wirth and Blanton, *C. mohave* Wirth and *C. (Selfia)* spp. were captured in light and vehicle traps operated routinely at 3 dairies between March and October 1979, but no adults of these species emerged from samples taken from larval sites in the dairy lagoons.

While previous surveys on larval sites have been concerned with numerous *Culicoides* species in various climatic zones (Williams 1956, Breeland 1960, Jones 1961, Hair et al. 1966, Battle and Turner 1970, Kardatzke and Rowley 1971, Braverman 1978), the present study concentrated on potential habitats for *C. variipennis* associated with geographically different endemic areas of BTV. Many of these areas provided a wide variety of manure-contaminated water sources which Wirth and Jones (1957) reported as the preferred habitats of some subspecies of *C. variipennis.*

The results of this study showed that in endemic BTV areas, gnat habitats were primarily man-made due to the adventitious construction of water impoundment facilities and poor waste-water management. In addition, of the 36 *C. variipennis* larval sites recorded, the greatest densities of gnats were repeatedly found in dairy waste-water lagoons.

A statewide dairy waste water survey conducted in 1980 showed that from 113 dairies sampled, 55% of the dairy lagoons supported early development of *C. variipennis* in the summer with dense larval production in 40% of these sources in the fall months (Loomis and O'Rourke 1982). Since most BTV isolations from cattle have been made between August and September, this seasonal occurrence matches peak production of *C. variipennis,* found to be the principal vector of this disease (Osburn et al. 1981). These results agree with those of Wirth and Bottimer (1956) which demonstrated comparatively high populations of *C. variipennis* in BTV endemic areas owing to this species preference for habitats associated with livestock.

Dairies which had good lagoons and efficient systems of water disposal were seldom found to support even light larval densities of *C. variipennis*. Properly constructed lagoons were steep-sided, devoid of vegetation, and contained a minimum amount of scum and/or floating solids. In addition, those dairies with primary cement holding tanks and/or liquid-solid separators were never found to support gnat development.

Although these results demonstrate that dairy lagoons are a permanent and conducive habitat for *C. variipennis*, while natural habitats are only marginally productive, other sources should not be overlooked. The diversity of larval sites found illustrates the opportunistic nature of this gnat to establish itself in a variety of ecological habitats.

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