# USE OF A PYRETHRIN LARVICIDE TO CONTROL *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) IN AN ALKALINE LAKE

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ABSTRACT. Laboratory bioassays with *Culicoides variipennis* larvae from Borax Lake, CA, indicated an $LC_{90}$ of 16.8 ppb for pyrethrins at 23°C. A field test in a pond adjoining the lake reduced adult *C. variipennis* emergence >99% for over 30 days after treatment at a rate of 131 ppb pyrethrins. Further small scale field tests indicated that *C. variipennis* could be controlled in the lake at a rate of 701 g/ha pyrethrins applied as a peripheral treatment. The first lake treatment resulted in a 99.3% decrease in the density of *C. variipennis* larvae along the shoreline. Four treatments resulted in a 98.5% decrease from non-treatment years in the number of adult cumulative insect-days during May and June.

## INTRODUCTION

*Culicoides variipennis* (Coquillett) is a biting pest of man and livestock. Females of the *C. variipennis* complex have been implicated as vectors of bluetongue viral disease in sheep and cattle (Luedke et al. 1967). Borax Lake, Lake County, California is a highly saline and alkaline lake, and has been a phenomenally productive source of these midges for many years. The lake lies in a natural basin with no outlet so winter rain and summer evaporation result in a wide range in depth (<1.0 to >4.0 m), area (<35 to >150 ha) and water quality parameters. Larval *Culicoides* habitat is largely restricted to a narrow band near the shoreline (Colwell 1981). Wetzel (1964) has described the limnology of the lake.

Traditional methods of control of *Culicoides* larvae have been ineffective at Borax Lake. Water management, recommended by Linley and Davies (1971), is not economically feasible. Borax Lake *Culicoides* larvae have high tolerance to many inorganic, organochlorine, carbamate and organophosphate insecticides (Apperson 1975, Kelson et al. 1980). The effectiveness of most insecticides is further reduced due to the action of the alkaline water on the active ingredients. Insect growth regulators (Apperson and Yows 1976) and *Bacillus thuringiensis* var. *israelensis* (Kelson et al. 1980) have also been ineffective.

Pyrethrins are non-persistent insecticides of low mammalian toxicity (Matsumura 1975) and are effective against some aquatic dipteran pests (Darwezeh and Mulla 1981). Preliminary tests with pyrethrins against *C. variipennis* larvae in Borax Lake water produced encouraging results. Barnard and Jones (1980) recommended larviciding to control *C. variipennis* populations in the overwintering period. At Borax Lake multiple treatments of the shoreline in early spring might control the larvae as the overwintering population migrates to shore to pupate. Larval control before significant spring emergence could also slow the reinfestation of the lake due to oviposition.

## METHODS AND MATERIALS

DETERMINATION OF LARVAL INSTARS. The larval instars of *C. variipennis* were separated on the basis of head capsule length measurements (n = 740) to the nearest 0.011 mm. The measurements were taken across the longest anterior to posterior line of the head capsule excluding mouthparts. Larvae preserved in 95% ethanol were used for this purpose.

BIOASSAYS. Measurements of a randomized subsample of the field-collected larvae used in the bioassay showed that 58% were fourth instars and the remainder were third instars. An eyedropper was used to add 15 larvae to 200 ml glass petri dishes holding 100 ml of stock solution to each treated dish. Controls received 10.0 $\mu$l of deionized water. Six concentrations of Pyrenone® Crop Spray (6.0% natural pyrethrins, from Fairfield American Corporation) were prepared by serial dilution of the concentrate. An Eppendorf pipette was used to add 10.0 $\mu$l of deionized water. All dishes were covered with lids and left in continual light. Four replicates of each of the six treatment concentrations and four control replicates were completed at 23±2°C. Since some larvae exposed to pyrethrins suffer knockdown but later recover, mortality was evaluated at 72 hr. Fully-developed pupae were counted as survivors. The $LC_{50}$ and $LC_{90}$ values were determined by a probit analysis (Finney 1971) using a Polo program (Robertson et al. 1980).

FIELD TESTS. Field tests were initiated in a levied pond 25.4 × 32.3 m with an average depth of 1.7 m. Prior to the treatment the pond and lake were connected by an opening 2.1 m wide by 1.2 m deep. This opening was closed with sandbags prior to the treatment.

A total of 3.105 liters of Pyrenone and 0.674 liters of Nalquatic® (aquatic pesticide carrier) were mixed with aged tap water to make a total volume of 11.6 liters. The mixture was applied on July 28, 1982 to the entire pond perimeter with a 7.5 liter hand sprayer. This treatment yielded a theoretical concentration of 131 ppb AI in the pond. Water temperature, pH and conductivity were measured in the lake and pond with a Presto-Tek DP-39 meter and total alkalinity was measured according to standard methods (Greenburg 1980). Larval *C. variipennis* populations were monitored by collecting sediment samples from the pond shoreline with a small core sampler (Kelson et al. 1980). Each cylindrical sediment sample covered a surface area of 7.1 cm$^2$ and had a depth of ca. 2.5 cm. Larvae and pupae were removed from the sediment for counting by flotation in a saturated solution of calcium chloride. Adult emergence was monitored by emergence traps placed at the mud-water interface. Each trap sampled a circular area of 17.0 cm$^2$. Emerging adults become entangled on the inner surfaces of the traps which were coated with Tanglefoot® (Kelson et al. 1980).

Eight control stations (on the lake) and eight treated stations were established. Sample location within each station was determined by use of a random numbers table. Six control and six treated stations were randomly selected and sampled on each sampling date. Immature populations were sampled on day 0 pretreatment and on days 1, 5, 8, 12 and 16 posttreatment. Adult emergence was collected from traps at six control and six treated stations at 2 days and at 2 hr pretreatment and 2, 5, 8, 12, 16, 19, 23, 26, 33, 37 and 43 days posttreatment. Numbers of live larvae and pupae and adult emergence at control and treated stations were compared by use of Mann-Whitney tests.

Further field testing was done along the shoreline of the lake. Treatment plots (137.2 m long with sampling stations near the middle) were delineated with markers. The shoreline area used was relatively flat with a maximum water depth of ca. 30 cm. Pyrenone was mixed with deionized water to give rates of 182, 463, 912 and 1838 g/ha of pyrethrins when applied by a rear mounted four nozzle boom sprayer on an Argo-8 All Terrain Vehicle (ATV). The ATV was operated at 3.2 km/hr during treatments. The sprayer gave a volume of 4.28 liters/min with a swath width of 3.7 m. The swath was directed at the shoreline water with 0.1 m extending above the mud-water interface. Larval mortality was evaluated by analysis of 2 shoreline sediment samples from each plot taken on day-1 pretreatment and on days 1, 4 and 7 posttreatment.

Shoreline treatments of borax lake. Core sample analyses were done at 1000 hr on both February 23, 1983 and March 14, 1983. Each core sample was taken to a depth of at least 9.0 cm with a K.B.® core sampler 50.8 cm long with an internal diameter of 5.08 cm. Two core samples were taken at each of shoreline, 0.25, 0.5 and 1.0 m depth stations. Offshore stations were 1.5, 3.5 and 8.0 m offshore, respectively. Each core of sediment was sectioned into 3.0 cm intervals (Anderson et al. 1980), and taken to the laboratory for analysis. Immature *Culicoides* were separated from sediment by flotation in a saturated solution of calcium chloride.

Four shoreline band treatments of the lake were completed in 1983. These were on March 15 and 18, April 8, April 22 and 26 and May 11. Two of the treatments required two treatment days due to inclement weather (wind or rain). All treatments were done with the ATV at a rate of 701 g/ha of pyrethrins.

At the time of the first treatment the shoreline of the lake measured 5,180 m. The treatment zone (0.1 m above to 3.6 m below the shoreline) comprised 1.92 ha of surface area. Maximum water depth in the treatment zone varied from ca. 30 to 250 cm. All treatments were done between 0800 and 1200 hr.

Shoreline sediment samples were taken from five stations around the lake perimeter on March 14, 18, 21 and 29, April 7, 11, 19 and 27 and May 9, 13 and 23, 1983 and analyzed for numbers of live larvae. Two New Jersey light traps were stationed in the same location as in previous non-treatment years (a residential area 1.6 km SE of Borax Lake) to monitor adult *C. variipennis* emergence. Light trap catches were collected weekly until the elapse of more than one generation time of *C. variipennis* after the last shoreline treatment of the lake.

## RESULTS AND DISCUSSION

Determination of larval instars. Figure 1 shows the head capsule lengths of *C. variipennis* larval instars. The mean length and standard deviation for first, second, third and fourth instars were 0.070±0.007 mm (n = 56), 0.114±0.009 mm (n = 149), 0.198±0.013 mm (n = 288) and 0.315±0.012 mm (n = 247), respectively. The ratios of the means of successive instars were consistent with Dyar's rule. Growth rates for several species of aquatic dipterans appear to be exponential rather than geometric (Nemjo and Slaff 1984). The Borax Lake larval head lengths fit the exponential equation, $Y=(0.0418)(1.6626)^X$, with a correlation coefficient of 0.99 indicating head lengths are useful in separating the instars of *C. variipennis*.

Bioassays. At 23°C the 72 hr $LC_{50}$ and $LC_{90}$ values of pyrethrins (formulated as Pyrenone) against *C. variipennis* larvae measured 3.98 and

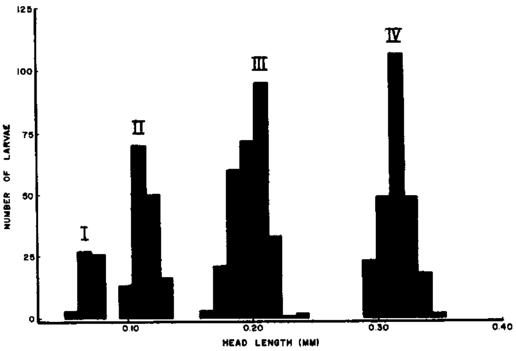

Fig. 1. Frequency of head capsule lengths of *C. variipennis* larvae from Borax Lake, CA. Roman numerals indicate larval instar designations.

Table 1. Mean numbers of *Culicoides variipennis* live larvae and pupae per cm$^2$ of sediment sample following formulated pyrethrin treatment of a pond.

| | Live larvae | | Pupae | |
|---|---|---|---|---|
| Day | Control | Treated | Control | Treated |
| 0 (pretreatment) | 4.74 | 3.40 | 29.95 | 10.96 |
| 1 | 8.97 | 0.07* | 18.90 | 0.07* |
| 5 | 4.01 | 0.05* | 50.80 | 0.12* |
| 8 | 4.39 | 0.75 | 10.14 | 0.07* |
| 12 | 2.35 | 0.96 | 18.92 | 0.00* |
| 16 | 2.00 | 8.12 | 8.08 | 0.05* |

* Number of treated organisms significantly lower than numbers of control organism by Mann-Whitney test ($p<0.05$).

16.84 ppb, respectively. Apperson (1975) reported 24 hr $LC_{50}$ and $LC_{95}$ data for eight chlorinated hydrocarbon and four organophosphate technical grade insecticides against *C. variipennis* larvae from Borax Lake. Larval tolerance to chlorinated hydrocarbons was high ($LC_{50}$ >1000 ppb). Among the eight organophosphate compounds tested, temephos was the most active ($LC_{95}$ = 72 ppb) and malathion was least active ($LC_{95}$= 1700 ppb). Comparison of these results indicates high susceptibility of Borax Lake *C. variipennis* larvae to pyrethrins relative to the other compounds tested. Similar bioassays have been conducted with the pyrethroids permethrin ($LC_{90}$ = 13 ppb) and fenvalerate ($LC_{90}$ = 28 ppb) (Colwell 1981). No reports are available to compare pyrethrins against *C. variipennis* larvae from other populations, however Holbrook (1982a) reported 24 hr bioassay data for permethrin ($LC_{95}$ = 71 ppb) and fenvalerate ($LC_{95}$ = 89 ppb) against colonized *C. variipennis* larvae.

Field tests. On September 28, 1982 (the day of the pond treatment) the water temperature was 26°C, pH 9.71, conductivity 18,800 $\mu$mhos/cm and total alkalinity 4,350 ppm. Following the pyrethrin treatment the mean number of live *C. variipennis* larvae per sediment sample in the pond was significantly lower than control samples 1 and 5 days posttreatment (Table 1). Numbers of live larvae were reduced 98% on those days when compared to pretreatment samples from the same locations. By 8 days posttreatment sediment samples from the pond contained live first instar larvae. Numbers of pupae were significantly lower at the treated pond on all sampling dates between 1 and 16 days posttreatment, averaging a 99.6% reduction over pretreatment samples at the same stations.

Adult emergence from the treated pond was significantly lower ($p<0.05$) than from the control pond on seven of nine sampling dates between 2 and 33 days posttreatment (Fig. 2). Treated pond emergence was reduced by 99.6% for 33 days when compared to control adult emergence. By 37 days posttreatment the emergence in the treated pond surpassed that of the control stations. The quick suppression of adult emergence and reduction of pupae per sediment sample both indicate high pupal mortality following the pyrethrin treatment. These results are indicative of excellent larval control and suppression of adult emergence for 1 generation (=536 degree days, Kelson et al. 1980) at a rate of 131 ppb pyrethrins in Borax Lake water.

Previous field trials involving Lethane® 384, methoprene, and *Bacillus thuringiensis* var. *israelensis* all failed to control adult emergence of *C. variipennis* in Borax Lake. Despite susceptibility to temephos in pond water bioassays, a field trial at 0.2 ppm failed to control *C. varii-*

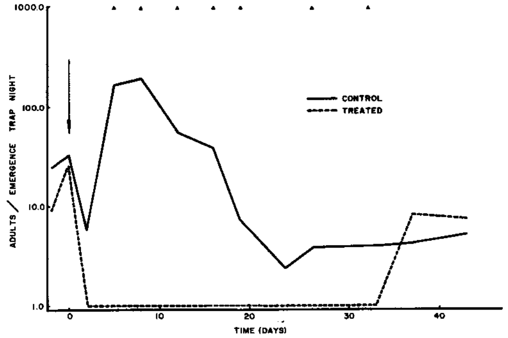

Fig. 2. Effects of formulated pyrethrin on adult emergence of *C. variipennis* from a levied pond. The triangle symbol indicates significant difference in control and treated emergence by a Mann-Whitney test ($p<0.05$).

*pennis* emergence (Kelson et al. 1980). Holbrook and Agun (1984) reported excellent control of *C. variipennis* larvae in Colorado with the same concentration of temephos in their field trials at sites polluted with animal manure.

Operationally a lakewide treatment with pyrethrins would not be economically possible. Colwell (1981) reported 89% of *Culicoides* larvae in Borax Lake were in water <0.2 m deep. This larval habitat zone forms a narrow band along the shoreline of the lake. Shoreline band treatments tests were done to determine if application of pyrethrins to the larval habitat area would be effective before the active ingredients diluted out into the body of the lake. At 1 day posttreatment an application rate of 182 g/ha of pyrethrins reduced numbers of live larvae at the shoreline by >96% (Table 2). No living larvae were found in shoreline sediment samples taken from plots treated at rates >182 g/ha pyrethrins. By 4 days posttreatment live first instar larvae were present in sediment samples taken from the plot treated at the highest rate. By 7 days posttreatment live fourth instar larvae were found in samples from each plot. Since fourth instars cannot develop from eggs in 7 days, the late instar larvae apparently migrated into the treated plots from deeper water or untreated areas of the lake. The pupal population in the treated plots was very low at the time of the treatments but plots treated at 182,463 and 1838 g/ha all showed reductions in numbers of pupae >90% by 1 day posttreatment. No pupae were found pretreatment in the plot treated at a rate of 912 g/ha. This test demonstrated that dilution of larvicide into the body of the lake was not a major obstacle to shoreline band treatments. Multiple treatments were planned to control larvae that initially survived in offshore locations.

Shoreline treatments of borax lake. Plans to begin shoreline treatments before the first pupation in 1983 were thwarted by nearly steady wet and windy weather during February and early March. Analysis of core samples, taken February 23 showed the overwintering population at that time consisted of 53.9% fourth instar, 39.2% third instar and 6.9% second instar larvae (n = 221). No pupae or first instars were recovered. By March 14, 1 day before the first shoreline band treatment, core sample analysis at the same stations showed 41.6% of the immatures recovered to be pupae (n = 346). The larvae were 85.4% fourth instars, 13.1% third instars and 1.5% second instars. Analysis of larval spatial distribution showed that only 4.0% of larvae in core samples taken at 1000 hr on March 14 were in the water column. Larvae in mud at the shoreline accounted for 69.3% of the population. More than 84% of the larvae recovered from the mud were in the top 3.0 cm of sediment but 0.5% were found as deep as 9.0 cm below the surface. Nearly 10% of the larvae collected were beyond the treatment zone (>3.6 m offshore). Just 3.5% of the larvae recovered were in the effective treatment zone (in the water column <3.5 m offshore), but Barnard and Jones (1980) reported considerable vertical and horizontal migration by *C. variipennis* larvae during a daily period in Colorado dairy ponds. Since pyrethrins are short-lived in the environment, primarily due to rapid photolysis (Casida 1980), timing of a treatment may be important in minimizing dosage rates. Preliminary observations from diel core sampling at Borax Lake indicate *C. variipennis* larvae are more prevalent in the water column in the evening and night hours. A nighttime pyrethrin treatment could minimize dosage rates by reducing photolysis and maximizing larval exposure.

Reductions averaging 96.8% of live larvae per shoreline sediment sample were noted after each of the first three treatments of the entire lake shoreline (Table 3). Larval populations recovered after each treatment but reached a lower peak density with each successive treatment. Offshore and deep water larval survival

Table 2. Effects of formulated pyrethrin (applied as shoreline band treatments) on density of *Culicoides variipennis* larvae recovered in sediment samples from experimental plots on Borax Lake. Treatment occurred on day 0.

| | Application rate (g/ha of pyrethrins) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 182 | | 463 | | 912 | | 1838 | |
| Time (days) | # live larvae | % change | # live larvae | % change | # live larvae | % change | # live larvae | % change |
| −1 | 7.75 | N/A | 31.27 | N/A | 2.39 | N/A | 6.20 | N/A |
| 1 | 0.28 | −96.4 | 0.00 | −100.0 | 0.00 | −100.0 | 0.0 | −100.0 |
| 4 | 0.14 | −98.2 | 1.76 | −94.4 | 0.00 | −100.0 | 0.14 | −97.7 |
| 7 | 7.75 | 0.0 | 16.69 | −46.6 | 3.10 | +29.7 | 0.59 | −92.1 |

N/A—not applicable.

Table 3. Mean numbers of live *Culicoides variipennis* larvae in sediment samples following pyrethrin treatments of the entire Borax Lake shoreline, 1983.

| Treatment date | Sample date | Live larvae/cm$^2$ | Days from treatment |
|---|---|---|---|
| | March 14 | 2.51 | 4 pre-first |
| | March 18 | 6.90 | 0.1 pre-first |
| March 15 and 18 | March 21 | 0.05 | 3 post-first |
| | March 29 | 0.82 | 11 post-first |
| | April 7 | 5.27 | 20 post-first |
| April 8 | April 11 | 0.35 | 3 post-second |
| | April 19 | 3.15 | 11 post-second |
| April 22 and 26 | April 27 | 0.07 | 1 post-third |
| | May 9 | 0.50 | 12 post-third |
| May 11 | May 13 | 1.15 | 2 post-fourth |
| | May 23 | 0.06 | 12 post-fourth |

was confirmed by the presence of pupae in shoreline sediment samples within 12 days of each treatment. Wind patterns created higher larval survival in some areas as did steep shoreline in others. Oviposition by adult females after the later treatments also contributed to larval population recoveries. In nontreatment years larval populations build up by June, averaging >15 larvae per cm$^2$ in shoreline sediment samples (Kelson et al. 1980). Sediment sample analysis on May 23, 1983 after the four pyrethrin treatments yielded a lakewide average of 0.06 live larvae per cm$^2$. Because of heavy spring rain Borax Lake water quality was best at the time of the last shoreline treatment. At that time the lake had a pH of 9.70, conductivity of 2,600 $\mu$mhos/cm and a total alkalinity of 1,120 ppm.

Analysis of New Jersey light-trap catches of *C. variipennis* adults from April 12 to June 12 (>1 generation time past the last treatment) showed the 1983 adult population during this treatment year was reduced by >98% when compared to adult catches at the same location during non-treatment years (Table 4). More important to local residents and ranchers near Borax Lake than percent control of adult *C. variipennis* in any given season, is the magnitude and duration of the adult population that survives a control measure. Ruppel (1983) proposed cumulative insect days as a measure of insect survival when subjected to a control measure. The term "insect days" is defined as:

$$\text{Insect days} = (X_{i+1} - X_i)\,[(Y_i + Y_{i+1}) \div 2]$$

Where $X_i$ and $X_{i+1}$ are adjacent points of time, and $Y_i$ and $Y_{i+1}$ are corresponding numbers of

Table 4. Mean numbers of *Culicoides variipennis* adults caught in New Jersey light traps 1.6 km SE of Borax Lake during control (1973–74) and treatment (1983) years.

| Month | Week | Control | Treatment | % reduction |
|---|---|---|---|---|
| April | 4 | 0.0 | 0.6 | N/A |
| May | 1 | 312.0 | 2.5 | 99.2 |
| | 2 | 364.0 | 3.0 | 99.2 |
| | 3 | 4,940.0 | 111.5 | 97.7 |
| | 4 | 4,576.0 | 276.0 | 94.0 |
| June | 1 | 5,512.0 | 193.8 | 96.5 |
| | 2 | 5,720.0 | 30.3 | 99.5 |
| | 3 | 11,232.0 | 35.5 | 99.7 |
| | 4 | 9,984.0 | 104.0 | 99.0 |

N/A—Not applicable.

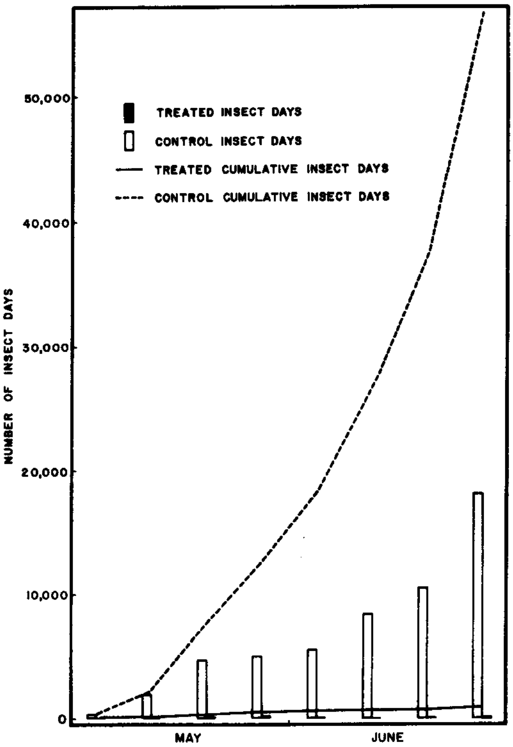

Fig. 3. Comparison of numbers of cumulative insect-days in a residential area near Borax Lake during control (1973–74) and treated (1983) years.

insects. Most complaints from local residents regarding the biting of Borax Lake *Culicoides* occur in May and June. During that period the number of cumulative insect days was reduced by 98.5% when compared to non-treatment years (Fig. 3).

The applicability of these findings should not be overgeneralized. *C. variipennis* larvae from Borax Lake are less susceptible to many pesticides than larvae from other regions (Holbrook 1982b). Borax Lake was fishless at the time of the treatments so under the conditions reported, pyrethrins appear to be an effective and safe larvicide for control of *C. variipennis*.

## ACKNOWLEDGMENTS

The authors are grateful for the technical assistance and cooperation of J. B. Haberthur, T. A. Nelson, and M. J. O'Rourke.

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