

## THE ATTRACTIVENESS OF CARBON DIOXIDE TO FEMALE *LEPTOCONOPS TORRENS* TNS., AND *L. KERTESZI* KIEFF.

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Recent studies have demonstrated the chemotropism of two species of *Leptoconops* to carbon dioxide under field conditions. Investigations concerning the attractiveness of chemical vapors to Ceratopogonidae are lacking in the literature, but host preferences and feeding habits are frequently reported (Kettle, 1962). Considerable information regarding the response of mosquitoes to chemical vapors is presented in a review by Clements (1963). Brown (1951) and Reeves (1951, 1953) relate the attractancy of culicine species to carbon dioxide under field conditions.

The development of an attractant trap was necessary for the evaluation of studies where adult *Leptoconops* density estimates were desired. Such an evaluative tool can then be utilized in studies of adult prevalence, location of breeding areas, assessment of insecticidal and cultural control, diurnal flight habits of the female, host preference, and flight range.

The carbon dioxide baited sticky cylinder trap to be described herein, attracts only those female gnats which are seeking a blood meal. Therefore, the nuisance or biting potential of the gnats can be estimated by the use of this trap, even though catches represent only a portion of the total adult female population. It seems reasonable that there may be a relationship between the numbers of adult female gnats which need a blood meal to complete their ovarian development and those gnats which represent the total female population. The initial and terminal stages of the emergence period may be an exception to this relationship. The attractant traps along with the half-square yard soil emergence traps previously employed, should give a good index of the

*Leptoconops* population. The usefulness of the attractant trap in population estimates during a season of low emergence is presented in this report.

This paper presents evidence to confirm that *L. torrens* and *L. kerteszi* are readily attracted to carbon dioxide gas and that their response is governed by certain physical factors. A description of the bait trap, standardization of baiting procedures, and potential uses for which this trap may be employed are discussed in detail.

**METHODS AND MATERIALS.** To conduct field evaluations of a population of adult *Leptoconops*, a trap was used similar to the one described by Dove (1932) and later used in modified forms by Burgess (1935), Bigham (1941), and Tinkham (1951). The soil emergence trap used by these workers was reduced in size to sample an area of one-half square yard. A collection jar was equipped with a cone type drinking cup that had the apex cut off. The gnats, seeking a light source upon emergence from the ground, enter the jar and are unable to return to the inside of the trap. After the trap was placed on the ground, a mixture of sand and soil was packed around the edge of the trap to prevent escape of adult gnats.

To measure airborne populations of *Leptoconops*, a sticky cylinder trap similar to the one described by Broadbent *et al.* (1948) was developed to incorporate the use of an attractant (Fig. 1). A piece of stove-pipe 12 inches long and 5 inches in diameter was supported on a 2 x 2 inch wooden pole 6 feet long. Two-inch nails were hammered into the pole at 12-inch intervals and bent over against the pole in order to provide a fastening for the top and bottom of the cylinder. The spac-

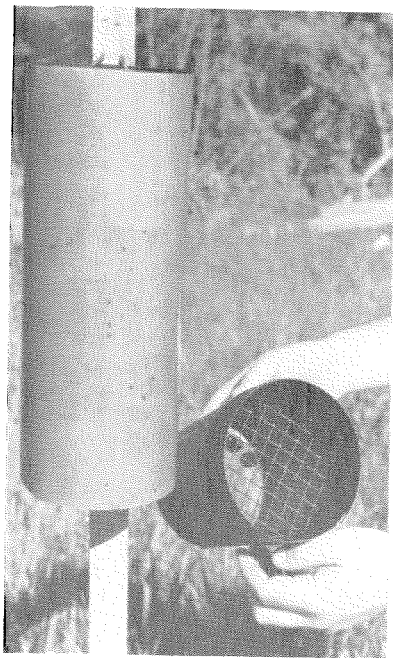


FIG. 1.—Carbon dioxide sticky cylinder trap.

ing of the nails allowed the workers to measure the population of *Leptoconops* at different levels above the ground. A 2mm layer of tree banding adhesive (Tanglefoot) was spread evenly on a sheet of kraft paper 12 x 18 inches with a 2-inch wide spatula. Another sheet of kraft paper was placed over this sheet and the two sheets were pressed together with the flat surface of a ruler.

This technique aided the workers to obtain a uniform coverage of the Tanglefoot, conserved time, and allowed the sheets to be prepared before they were transported to the field. If the paper was cut so that its length was slightly greater than the circumference of the cylinder, the adhesive quality of the Tanglefoot held the paper in place. Two inches from the bottom of the cylinder a piece of hardware cloth ( $\frac{1}{2}$  inch mesh) 5 inches in diameter was soldered to the inside wall of the cylinder, to provide a flat surface on which to place a selected test material for *Leptoconops* attraction.

Several materials suspected of being capable of attracting adult *Leptoconops* were selected to be used in the sticky cylinder trap studies. They were L-Arginine-L-Glutamate, Glycine, Sebacic acid, Lactic acid, Cholesterol, Estriol, Creatinine, Methyl Caprate, Urea, Tributyrin, Yeast Hydrolysate (enzymatic), Soy Hydrolysate (enzymatic), Lactalbumin Hydrolysate (enzymatic), Blood Hydrolysate (enzymatic), Casein Hydrolysate (enzymatic), Protein digest proteoses (Bacto-Protone), and Casein digest, pancreatic (Bacto-Casitone). The materials were tested singly and in multiple combinations for their attractiveness to adult *Leptoconops*. One percent solutions (100 ml.) of the materials to be tested were placed in the sticky cylinder traps. In addition, other trials were conducted in which filter paper was saturated with a 1 percent solution of each of the above materials. The filter paper was moistened continuously from below by a wick immersed in distilled water. A 10 percent solution of soluble dried blood was tested in a similar manner.

The carbon dioxide was obtained from dry ice. A package with 2 to 3 pounds of dry ice wrapped in several thicknesses of newspaper was placed in the cylinder and an opening was cut into each end of the package to provide for the escape of carbon dioxide. The workers noted that when the relative humidity was above 60 percent, condensation formed on the kraft paper. This factor reduced the number of *Leptoconops* collected from the traps at a time when the workers were well aware of the large number of gnats in the vicinity of the sampling area. Therefore, to reduce condensation problems, the workers found it was necessary to insulate the inside of the trap with an ice cream bag.

In addition to the dry ice-baited traps a small tank of carbon dioxide (38-pound hospital type tank), controlled by a standard gas pressure regulator calibrated in pounds per square inch with a connecting hose leading to a sticky cylinder trap, was used to supply the attractant to assess the adult female *Leptoconops* population.

While the tank-baited trap was successful in attracting adult females, and was free of condensation problems during periods of high relative humidity, there were a number of disadvantages associated with its use. The chief disadvantage would be the prohibitive cost of sampling gnats over a wide area.

It became apparent during these studies that a sample of dry ice dissipated more rapidly as the wind velocity increased. Therefore, if a worker wanted to use this trap as an index to assess the population of adult *Leptoconops* it would be valuable to know the approximate effective duration of the dry ice. To determine this, the rate of sublimation of dry ice was studied under varied temperatures and wind speeds in a draft-free room. A 12-inch table fan was directed at a 1-pound sample of dry ice elevated on blocks of wood to correspond to the shaft height of the fan. The varied wind speeds (0, 3, 5, 7, & 9 m.p.h.) were measured by the use of a cup anemometer (U. S. Forest Service Type, Chisholm 2B3C) mounted on a block of wood and elevated so that the height of the cups corresponded to the height of the sample of dry ice. Each sample was weighed at 15-minute intervals

and the weights were recorded for the duration of the test. A Harvard Trip double beam balance proved to be ideal for weighing the samples quickly. A thermostatically controlled 1650 watt electric heater enabled the workers to conduct the sublimation studies at 70°, 80°, and 90° F. Both wind and temperature independently increased the sublimation of the samples of dry ice, as shown in Table 1. However, wind caused the most pronounced increase in the rate of sublimation as is noted by the comparison of the 0 and 9 m.p.h. wind speed trials at 90° F.

In the field, the wind speed was checked at 15-minute intervals by a cup anemometer mounted on a pole 4 feet above the ground. This corresponded to the height of the sticky cylinder traps. The traps, located at random in a known breeding area, were positioned a minimum of 15 feet apart so as to avoid contamination between the traps. A systematic removal of the gnats from the sticky paper was accomplished with a spatula as each hourly assessment was made of the attractant and check traps. Then unwanted insects were wiped from the adhesive surface in preparation for the next hourly assessment.

TABLE 1.—Sublimation of dry ice effected by specific physical factors.  
(One pound (453.6 grams) of dry ice used for each trial.)

Wind Speed (miles/hour)	Temperature (° F.)	Gram weights of dry ice after successive 15-minute time intervals						
		1	2	3	4	5	6	7
0	70	289.3	183.0	112.8	63.8	26.9	3.6	0
	80	275.0	165.8	93.6	40.6	5.3	0	..
	90	269.0	155.1	75.3	18.8	0	..	..
3	70	207.7	76.4	11.0	0	..	..	..
	80	201.9	50.4	0.5	0	..	..	..
	90	160.5	3.1	0	..	..	..	..
5	70	131.4	6.6	0	..	..	..	..
	80	128.8	0	..	..	..	..	..
	90	40.5	0	..	..	..	..	..
7	70	47.3	0	..	..	..	..	..
	80	6.2	0	..	..	..	..	..
	90	2.7	0	..	..	..	..	..
9	70	21.8	0	..	..	..	..	..
	80	1.2	0	..	..	..	..	..
	90	0	..	..	..	..	..	..

Normally the kraft paper may be utilized for several hourly periods. The workers noted that it was essential to assess the hourly collection as rapidly as possible since their presence in the vicinity of the traps produced spurious results.

Relative humidity was measured by a sling psychrometer (20° to 110° F.) graduated to 1.0 degrees. Field and laboratory temperatures were obtained from a mercury thermometer (0° to 220° F.), graduated to 2.0 degrees.

**RESULTS.** Sampling adult *L. torrens* has previously been accomplished through the use of soil emergence traps and hand catches. While the soil emergence trap is an effective method of collecting emerging adults it has disadvantages which limit its use. In control investigations where large acreages are encountered, the number of traps necessary to gain some assessment of the control may prove to be unwieldy as well as costly. Furthermore, the workers are uncertain as to the influence an insecticide, applied to the soil and vegetation, may have upon emerging gnats confined within an emergence trap. In an open field, the newly emerged adults may normally reject treated areas in favor of un-

treated areas. On limited chemical control plots used to assess the effect of a given insecticidal formulation on gnat emergence, the emergence trap is indispensable. This trap is extremely valuable in cultural control investigations, diapause studies, and estimates of adult prevalence.

An estimate of the adult black gnat densities over a 5-year period in a breeding area near Dixon, California is given in Figure 2. The extent of emergence for a given area has recently been attributed to the quantity and duration of precipitation during the period after oviposition and prior to emergence (Whitsel and Schoepfner, 1964).

During the 1964 emergence period, initial studies were conducted to test separately and in combination a group of biochemical substances of mammals which were thought to act as attractants for *L. torrens*. From the previously listed materials only carbon dioxide was found to be attractive to female *L. torrens*. Subsequently, the workers found that carbon dioxide served to attract female *L. kerteszi* as well as *L. torrens* as reported in Table 2. Quite by accident, the workers noted that in a single evening 110 *Culicoides variipennis* were caught on one trap.

The apparent increased degree of attractiveness for *L. kerteszi* over *L. torrens* to carbon dioxide was due to the greater density of the adult *L. kerteszi* experienced at the Pebble Beach site during the sampling period. The population of *L. kerteszi* appears to be approximately four times greater than the population of *L. torrens* when sampled under similar conditions, as indicated by Table 2. The extremely reduced number of adult *L. torrens* captured in emergence ("check") traps during 1964 at the Dixon site is shown in Figure 2. In many areas known to be infested with *L. torrens* in previous years, their absence was conspicuous during the emergence period of 1964.

During seasons of low emergence levels, the usefulness of the attractant trap is demonstrated when we note that at the *L. torrens* breeding site soil emergence traps

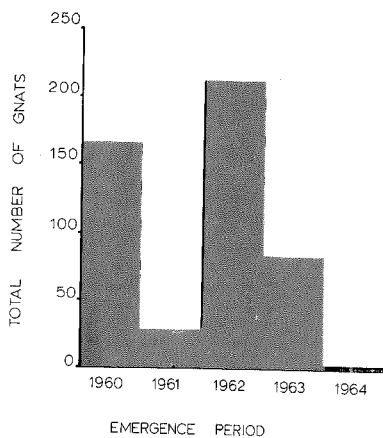


FIG. 2.—Yearly densities of adult black gnats (*L. torrens*) from 10 preselected control traps near Dixon, California.

TABLE 2.—Total numbers of female *Leptoconops* collected from sticky cylinder traps.

Number of trap hours	<i>Leptoconops kerteszi</i>			Percent check of total	Number of trap hours	<i>Leptoconops torrens</i>		Percent check of total
	CO <sub>2</sub> baited traps	Check* traps	Percent check of total			CO <sub>2</sub> baited traps	Check* traps	
20	641	27	4.0		48	386	10	2.5

\* (Unbaited traps)

did not yield a single gnat while the carbon dioxide baited traps collected 386 gnats in 48 trap hours.

Various physical factors were found to decrease the number of gnats collected on the carbon dioxide baited sticky cylinder traps. Increased wind velocity acts to reduce the airborne adults regardless of other physical factors favorable to flight activity. At the sampling stations, afternoons are generally characterized by increased wind velocities, reflected in a reduction in airborne gnats (e.g. *L. kerteszi* in Figure 3). Flight of *L. torrens* and *L. kerteszi* adults is greatly reduced when the mean wind velocity exceeds 8 m.p.h. In contrast, Davies (P.C., 1964) reports that in Jamaica *L. bequaerti* bites readily at wind speeds up to 13 m.p.h. The influence of wind on the flight of *L. torrens* is shown in Figure 4. Four hourly collections of *L. torrens* for each of four slightly different wind speeds, at temperatures considered nearly optimum for flight, reflect a decrease in adult numbers with only a very slight increase in average wind speed (Fig. 4).

There is some indication that air temperatures over 100° F. may reduce the biting activity of *L. torrens*. Both species of *Leptoconops* become vicious biters when the air temperatures rise to near or above 70° F. It is doubtful if air temperatures are high enough at any time at Pebble Beach to limit the flight activity of *L. kerteszi*.

The density of adult gnats normally increases during the daylight hours, and under favorable conditions are most prevalent from 11 a.m. to 3 p.m. This apparent increase in numbers experienced during

the day is probably a response by the gnat to the usual daily temperature pattern. The biting activity of *L. torrens* has been recorded when the relative humidity

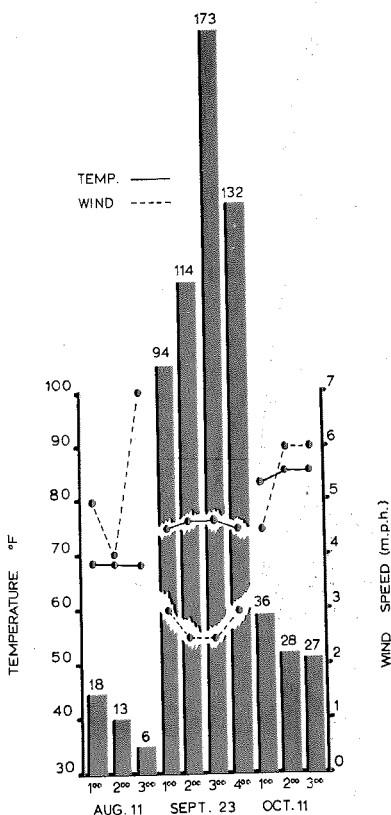


FIG. 3.—The effect of varied meteorological conditions upon numbers of *Leptoconops kerteszi* (figure above bar) collected at hourly intervals on carbon dioxide baited sticky cylinder traps in 1964.

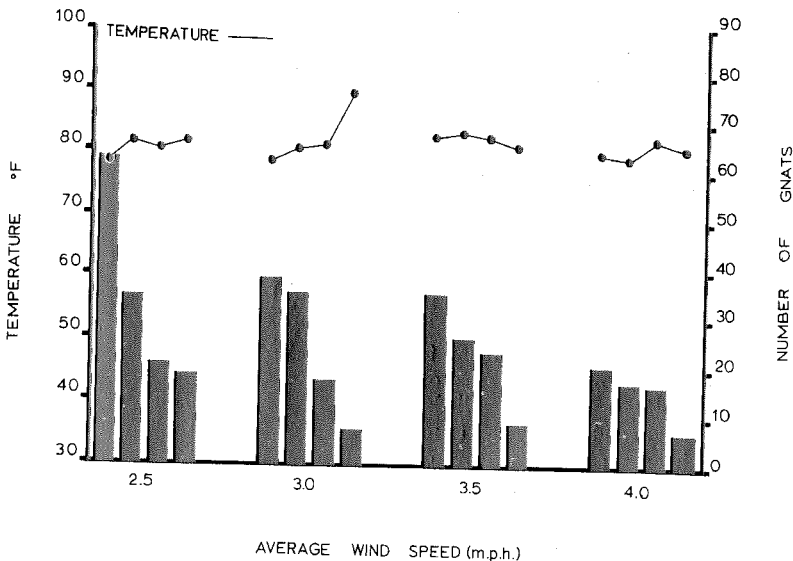


FIG. 4.—The effect of wind on the number of *Leptoconops torrens* collected hourly from carbon dioxide baited sticky cylinder traps in 1964.

ranged from 18 to 62 percent. A range of 24 to 70 percent relative humidity has been recorded when *L. kerteszi* has been biting at the Pebble Beach breeding area. The latter species is known to occur in far more arid environments. The wider variations in relative humidity do not appear to influence flight activity when other physical conditions remain favorable.

The number of female gnats caught on the baited traps was greatly affected by the sublimation rate of the dry ice. Since quantitative concentrations of carbon dioxide have not been field tested in the sticky cylinder trap it is not possible to say what quantity of carbon dioxide is preferred by the two *Leptoconops* species. The rate of release may very likely be correlated with the amount of carbon dioxide discharged by the gnats' preferred host. The rate at which carbon dioxide was discharged from the dry ice during field testing may well have been excessive and may have acted to discourage gnats from alighting on the trap. The quantity of carbon dioxide released from a 1-pound sample of dry ice over a 1-hour period when the air

temperature ranged between 70 and 90° F. and the average wind velocity ranged from 0 to 3 m.p.h. would be equivalent to that discharged by 8 to 12 humans.

**DISCUSSION.** It was apparent to the workers that modifications of the sticky cylinder trap would improve its usefulness in further field studies of *Leptoconops*.

The workers feel that 2 pounds of dry ice is a suitable quantity to be used as an attractant for *Leptoconops* under varied field conditions having a 1-hour duration. The ice may be satisfactorily insulated to prolong the effective duration of the attractant, if wrapped with newspaper. A small opening should be cut in the top and bottom of the package to allow escape of the gas. It is preferable to divide the dry ice into a small number of pieces.

During increased wind speeds, it was noted that a greater number of gnats were collected on the sheltered side of the trap. In order to demonstrate the effect of wind on the dispersion of gnats on the sticky cylinder trap, the workers intend to modify this trap in such a manner that it will be directed constantly into the wind. The

workers are cognizant of the need to reduce the time spent when counts are made during periods when large numbers of gnats are encountered. Modifications which would give a representative sample of the entire collection may be achieved by horizontal banding of the adhesive on the kraft paper.

The attractant may be supplied by a carbon dioxide tank to facilitate the use of this trap in remote areas. The initial cost of a sizable project for detection or assessment of a gnat population may make the use of carbon dioxide tanks prohibitive. In addition, loss of these unattended units in the field would be costly.

Studies of airborne *Leptoconops*, formerly limited by the customary sampling procedures, will be augmented by the use of this trap. In addition to this obvious advantage, the trap is both inexpensive and easy to maintain.

The employment of this trap will permit the easier location of breeding areas and make possible the assessment of large scale control programs. It has been noted that in breeding areas which usually show low adult prevalence, the carbon dioxide sticky cylinder trap gives some degree of assessment while the soil emergence trap is ineffective. The inability of the latter trap to yield gnats under these circumstances is due to its limited sampling area.

With the carbon dioxide baited sticky cylinder trap, it is possible to release marked female *Leptoconops* from a designated location and recapture them at established distances from the point of release. Studies of the influence of certain meteorological conditions upon flight activity will be aided by the employment of this trap. Previously, hand catches and biting counts were our only tools for assessment of airborne behavior.

The fact that the gnats are attracted to carbon dioxide is important in that the quantitative level of gas most attractive to the *Leptoconops* may suggest their host preference habits.

One distinct disadvantage of this trap is

its inability to collect adult females in all physiological phases. The trap depends largely on its ability to attract those female gnats which are at a stage in their ovarian cycle when a blood meal is sought. The male population is not sampled by this trap.

Although the assessment of large scale control programs can be readily accomplished with this trap, its use in small plot studies is difficult because of the trap's ability to attract female gnats from beyond the plot area.

Since varied meteorological conditions have been shown to influence gnat collections, it is important that only samples collected under similar environmental conditions be compared.

As previously discussed, trap counts may be influenced by the presence of the observers.

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