lege, Alaska for helpful suggestions and verification of mosquito identifications, and to AHRC for the loan of a microscope and other equipment. Thanks are also due Mr. G. I. Goldschmidt, Chief, Environmental Health Services Branch, Office of Environmental Health, U. S. Public Health Service, Anchorage, Alaska for program support which made the investigative study possible.

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EFFECT OF AMOUNT OF CO_2 ON COLLECTION OF TABANIDAE IN MALAISE TRAPS[1, 2]

R. H. ROBERTS

Entomology Research Division, Agr. Res. Serv., U.S.D.A., Stoneville, Mississippi 38776

ABSTRACT. In three studies to determine the differential response of Tabanidae to incremental increases in CO_2 (rates of 0, 100, 500, 1000, 1500, and 2000 ml/min.) used as bait in Malaise traps, the response proved to be species specific though the increases in catch were proportionately less at rates above 1000 ml/min. In all, 12 of the 20 species collected were present in sufficient numbers for statistical analyses. Four of these, *Chrysops flavidus* Wiedemann, *Tabanus fuscicostatus* Hine, *T. lineola* F., and *T. subsimilis* Bellardi, were present in all three studies; the other 8 were present in sufficient numbers in only one or two of the three studies.

The statistical analyses showed significant differences for rates of CO_2, dates of collection, and trap site while the coefficient of variation for each species analyzed indicated a high degree of variation.

In my previous paper (Roberts, 1970), I reported the results of a preliminary study of the attractiveness of amounts of CO_2 to Tabanidae. The data indicated that a linear relationship probably existed between the CO_2 released and the number of Tabanidae collected, but several aspects of this study prevented a critical analysis of the relationship. The present paper presents the results of a new study of that relationship conducted in 1969 in the Delta Branch Experimental Forest located about 1 mile north of the Delta Branch Experiment Station, Stoneville, Mississippi.

MATERIALS AND METHODS. The Experimental Forest is a tract of 2580 acres (Putnam and McKnight, 1949), which is drained from west to east by two canals. One is located at the southern boundary of the forest and the other is 1 mile north. The forest area extends another mile north of the second canal. The east-west

[1] In cooperation with the Delta Branch of the Mississippi State University Agricultural and Forestry Experiment Station, State College, Mississippi 39762.

[2] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the U.S.D.A.

boundaries are about 2 miles apart, and two north-south gravel roads, one on the west boundary and the other 1.25 miles east, connect the two canals. Halfway between the canals, an east-west gravel road connects the two north-south roads. Dirt roads extend to other portions of the forest and along the canal banks which are passable only in dry weather.

Two patterns of six trap sites were used in the three studies. The first was linear: 3 Malaise traps (Townes, 1962) constructed of natural saran screen were placed on the east side of the eastern north-south road 0.1 mile apart starting at and going south from the second canal. Three other traps were placed on the west side of the road 0.1 mile apart and 0.05 mile from the traps on the east side of the road. The 3 traps on the west side were identified (from south to north) as 1, 3, and 5, and those on the east side were identified as 2, 4, and 6. Trap 1 was 0.35 mile from Trap 6.

The second pattern had the 6 traps dispersed. Three were located on roads and 3 on the banks of the drainage canals: Traps 1 and 6 were located on the south canal 1.25 miles apart; Trap 3 was located on the north canal 1 mile north of Trap 1; Trap 2 was located on the east north-south road halfway between Traps 1 and 3; Trap 5 was located on the west north-south road ½ mile north of Trap 6; and Trap 4 was located on the east-west road halfway between Traps 2 and 5.

The CO_2 used in the tests was released from 50 lb. tanks by single-stage regulators and adjusted with needle valves to the desired rate of flow through Gilmont® compact flowmeters. The gas was released about 3 ft. above ground level on the center pole of the trap. Rates of release were 0, 100, 500, 1000, 1500, and 2000 ml/min. A randomized Latin square design was used to assign the CO_2 rates so that all rates were tested at each trap site.

Collections in the first two studies were made for a 24-hr. period beginning at 10 a.m. (Central Daylight Time) and terminating at 10 a.m. the following day. The first study was made May 21–29 (traps placed in the linear pattern) and the second study June 11–19 (traps placed in the dispersed pattern). Then a third study (dispersed pattern) was made July 7–16. However, collections from this latter study were made only during a 6-hr. period from 5 a.m. (CDT) to 11 a.m.

RESULTS AND DISCUSSION. The numbers of females of each species of Tabanidae collected in the three studies are shown in Table 1 for the 1000 ml/min. release level only. A total of 20 species—1 *Chlorotabanus*, 2 *Chrysops*, 2 *Hybomitra*, 1 *Leucotabanus*, and 14 *Tabanus*—were collected. However, only 12 species were collected in sufficient numbers for statistical analyses and of this number, only 4, *C. flavidus*, *T. fuscicostatus*, *T. lineola*, and *T. subsimilis*, were present in sufficient numbers in all three studies. The other 8 species were collected in adequate numbers in only one or two of the three studies. In Figs. 1 through 7, the ratio of the number collected with CO_2 to the number collected without CO_2 is shown on semi-log graph to demonstrate the proportional increases related to the levels of CO_2.

In general, the analyses of variance of the differences in numbers trapped with varying amounts of CO_2 were either statistically significant or highly significant, with some notable exceptions. For example, in the first study, the differences were not significant for *C. flavidus*, but in the other two studies, the differences were significant; the differences were significant for *T. fuscicostatus* only in the first study; the differences for *T. lineola* were highly significant in the first two studies and not significant in the third study; and the differences for *T. proximus* were not significant in the second study but were highly significant in the third study. Statistical definition between the rates of CO_2 release as determined by Duncan's multiple range test for all species analyzed was not sharp due to the high degree of variation that occurred. Graphically, a response to changes in CO_2 levels is

Table 1.—Numbers and species of Tabanidae collected at the apparent optimum release level of CO_2 in each of the 3 studies (total of 6 Malaise trap collections).[a]

Species	Numbers collected at release level of 1000 ml/min.					
	24-hour collections				6-hour collection	
	Linear May 21–29	C (%)[b]	Dispersed June 11–19	C (%)	Dispersed July 7–16	C (%)
Chlorotabanus crepuscularis (Bequaert)	1	....	122	62.2	11	....
Chrysops						
callidus Osten-Sacken	4	....	0	....	0	....
flavidus Wiedemann	1602	73.3	516	53.9	100	81.4
Hybomitra						
hinei wrighti (Whitney)	0	....	0	....	0	....
lasiophthalma (Macquart)	528	67.3	3	....	0	....
Leucotabanus annulatus (Say)	0	....	0	....	1	....
Tabanus						
abdominalis F.	0	....	188	66.7	920	59.3
americanus Forster	2	....	34	88.3	3	....
atratus F.	24	55.6	9	99.6	3	....
cymatophorous Osten-Sacken	0	....	5	....	0	....
equalis Hine	10	....	182	81.0	0	....
fuscicostatus Hine	738	33.4	4676	134.4	348	85.7
lineola F.	456	40.1	1866	47.7	10	84.8
mularis Stone	0	....	16	....	1	....
proximus Walker	0	....	44	72.4	43	71.7
stygius Say	1	....	2	....	0	....
subsimilis Bellardi	6576	22.5	1420	33.3	150	46.7
trimaculatus Palisot de Beauvois	3	....	1	....	0	....
venustus Osten-Sacken	5	....	7	....	0	....
wilsoni Pechuman	228	44.4	280	50.6	0	....

[a] Complete collection data for each study for all rates of CO_2 release will be furnished by the author upon request.
[b] Coefficient of variation.

shown by all species in each study that were collected in sufficient numbers for analysis. The response to increasing levels of CO_2 was specific for each species within each study, but the response of individual species varied between studies.

The greatest proportional increase per rate of CO_2 was shown by *T. americanus* followed by *T. atratus, C. crepuscularis, T. abdominalis* and *T. equalis.*

Generally, the numbers of a species collected at CO_2 release rates of 1000, 1500, and 2000 ml/min. were not significantly different. The greatest proportional increase occurred at rates of release of 100 and 500 ml/min. The approximate level of greatest response appeared to be the 1000 ml/min. rate. However, the actual level of greatest response is probably influenced by how far from the release point the CO_2 is detected by the flies and by the number of flies present in the area that can respond; both appear to be species related.

The dates of collection for *C. crepuscularis, H. lasiophthalma, T. abdominalis, T. subsimilis,* and *T. wilsoni* showed a statistically significant difference whenever sufficient numbers were collected for analysis, but those for *T. lineola* showed a highly significant difference only in the first study, and those for *T. proximus* showed a highly significant difference only in the second study. These data are interpreted to indicate that the populations of these species were in a state

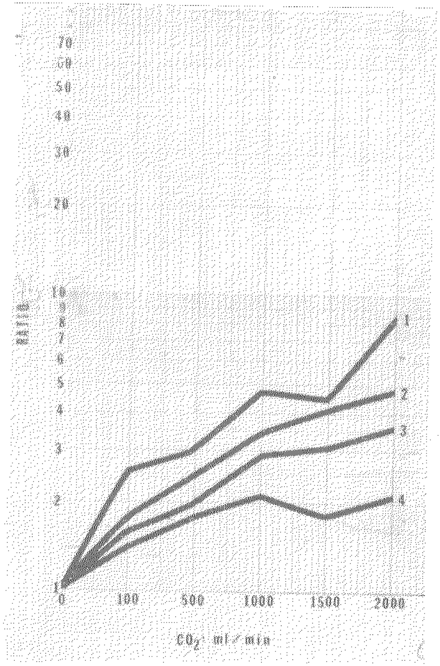

Fig. 1.—Effects of CO_2 on May 21–29 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. T. *atratus*, 2. *C. flavidus*, 3. *T subsimilis*, 4. *T. fuscicostatus*)

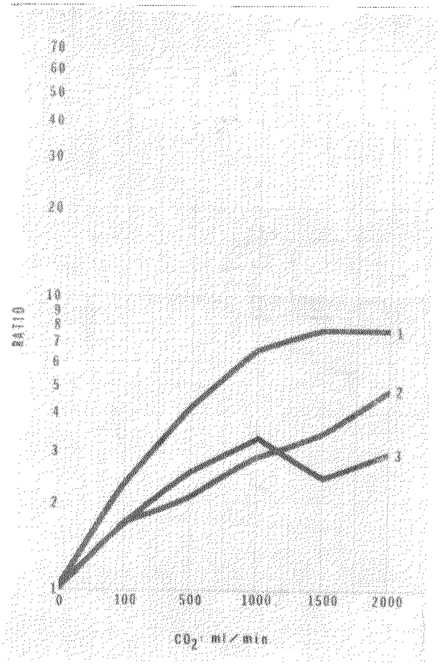

Fig. 2.—Effects of CO_2 on May 21–29 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. *H. lasiophthalma*, 2. *T. lineola*, 3. *T. wilsoni*)

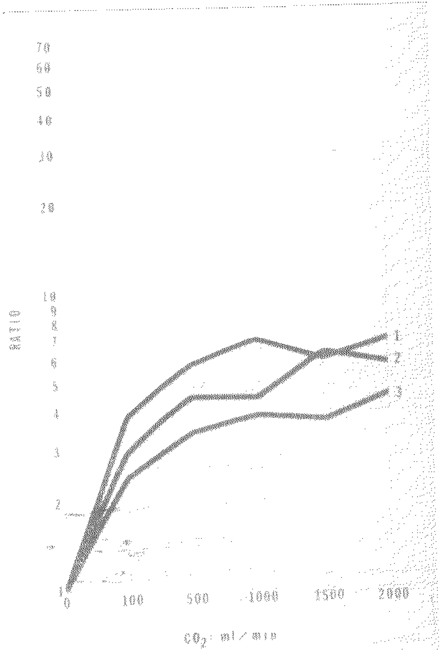

Fig. 3.—Effects of CO_2 on June 11–19 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. *T. lineola*, 2. *T. proximus*, 3. *C. flavidus*)

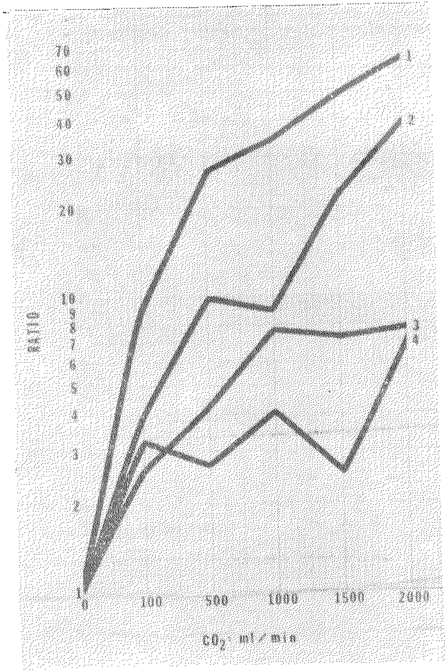

Fig. 4.—Effects of CO_2 on June 11–19 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. *T. americanus*, 2. *T. atratus*, 3. *T. abdominalis*, 4. *T. fuscicostatus*)

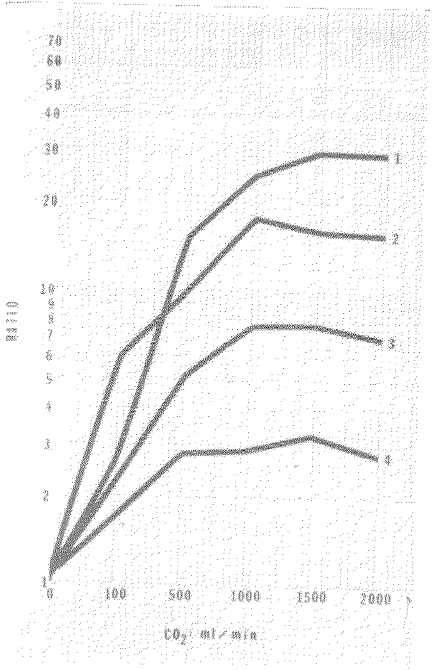

FIG. 5.—Effect of CO_2 on June 11–19 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. *C. crepuscularis*, 2. *T. equalis*, 3. *T. subsimilis*, 4. *T. wilsoni*)

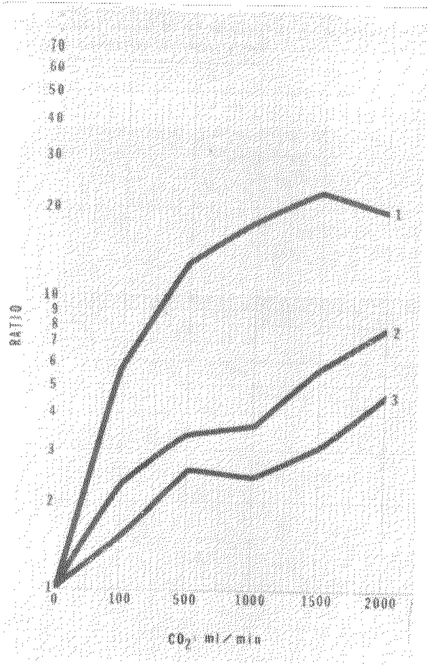

FIG. 6.—Effect of CO_2 on July 7–16 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1 *T. abdominalis*, 2. *T. subsimilis*, 3. *T. fuscicostatus*)

of flux during these collection periods, while the populations of the other species were constant.

Trap location played a role in the number of flies collected. In the linear pattern, Traps 1 and 6 collected significantly more flies of all species except *H. lasiophthalma* indicating that flies were entering the road flyway at the junction with the east-west road and the canal rather than infiltrating into the road from the surrounding forest area.

In the two studies with the dispersed trap site pattern, Traps 2, 4, and 5 (located on the roads) collected significantly greater numbers of 8 species than Traps 1, 3, and 6 (on the canals). However, the numbers of *T. americanus, T. atratus,* and *T. wilsoni* collected in the 6 traps did not differ significantly. Trap 5 collected greater numbers of *T. abdominalis* and *T. fuscicostatus;* Trap 2 collected more *C. crepuscularis* and *T. proximus;* Traps 2 and 4 collected more *C. flavidus;* and Traps 2 and 5 collected more *T. lineola.* The numbers of *T. equalis* and *T. subsimilis* collected were statistically equivalent at all 3 road sites.

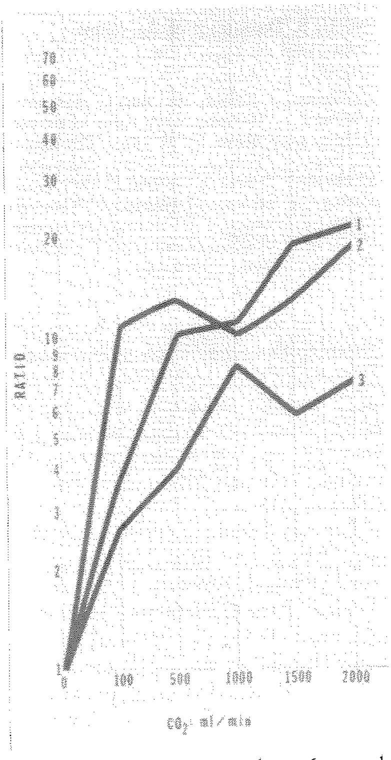

FIG. 7.—Effect of CO_2 on July 7–16 trap collections of Tabanidae as shown by the ratio:

$$\frac{\text{No. collected with } CO_2}{\text{No. collected without } CO_2}$$

(1. *T. proximus,* 2. *T. lineola,* 3. *C. flavidus*)

Thus, the statistical analyses of the numbers collected in each collection period showed highly significant differences for CO_2, date of collection, and trap site. However, the most interesting statistical value was the coefficient of variation, which indicated a high degree of variability in species response to CO_2, trap location, and date of collection. Factors that might contribute to this variation are (1) the difference of response of flies in more than one physiological state, (2) the effect of environmental factors such as temperature and humidity, (3) the effect of air movement on the distribution of CO_2, (4) the changes in population due to the normal seasonal cycle or to depletion by trapping, (5) the location of traps with respect to sites of adult emergence, and (6) variations in the delivery of CO_2 due to temperature effect on the regulators. Periodic checks of the regulators during each collection day showed that the output tended to vary about 10 percent above and below the level originally set; outputs increased during the afternoon, and decreased during the night. This effect of temperature on the regulators was one reason for the third study made July 23–30 from 5–11 a.m., the period when the least fluctuation occurred. However, shortening the collection period did little or nothing to reduce the variation.

Several conclusions can be drawn from this study: (1) CO_2 is an attractant for horse flies, while each species has a definite individual response to CO_2; (2)

most species are not equally distributed throughout the forest but tend to be more concentrated in flyways associated with the roads; (3) in studies of potential attractants, traps should be at least 0.3 mile apart to prevent undue trap competition; (4) collection periods of at least 6 hrs. appear feasible for studies of tabanid attractants.

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A TWO-YEAR SURVEY TO DETERMINE THE INCIDENCE OF A MERMITHID NEMATODE IN MOSQUITOES IN LOUISIANA

J. J. PETERSEN AND O. R. WILLIS [1]

Entomology Research Division, Agr. Res. Serv., U. S. Department of Agriculture
Lake Charles, Louisiana 70601

ABSTRACT. When a 27-month survey was made to determine the activity of the mermithid *Reesimermis nielseni* Tsai and Grundmann in mosquito larvae in Louisiana, 13 of the 19 species of mosquitoes present proved to be hosts, and 52 percent of the *Anopheles crucians* Wiedemann, 37 percent of the *Culex erraticus* (Dyar and Knab), and 30 percent of the *Uranotaenia sapphirina* (Osten-Sacken) were parasitized. In southwestern Louisiana, the parasite was active from April to November when mean water temperatures were above 65° F. Low densities of host, loss of water from the habitats, and physical and chemical changes in the habitats were factors reducing the incidence of parasitism. Thus, the number of mosquitoes of any species captured and the level of infection varied greatly, both for a given species and between species.

Numerous authors have reported the incidence of mermithid nematodes in mosquitoes, but most of these reports have been limited to one or at most to a very few observations. These studies generally indicated that when mermithids were present the incidence of parasitism was high, but no long-term observations have been made to determine fluctuations in parasite activity or in the long-term incidence of parasitism.

An earlier study of the bionomics of *Reesimermis nielseni* Tsai and Grundmann in Louisiana included periodic observations of the incidence of parasitism in several species of mosquitoes and reported natural parasitism in 13 species (Petersen *et al.*, 1968). Also, studies with *R. nielseni* were made in the laboratory to determine the potential of various species of mosquitoes as hosts (Petersen *et al.*, 1969) and the effects of the ratio of nematodes to hosts, volume and salinity of the water, and age of the larvae on the incidence of parasitism (Petersen and Willis, 1970).

The present study was made at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory, Lake Charles, Louisiana from October 1968 to January 1971 to determine the effects of environmental factors on the incidence of parasitism by *R. nielseni* in mosquitoes; to measure the yearly fluctuations in parasite activity; and the effectiveness of species of mosquitoes as natural hosts of this parasite. This mermithid (previously reported as an undescribed species of *Romanomermis*) has been tentatively identified as

[1] In cooperation with McNeese State University, Lake Charles, Louisiana 70601.