early stages of British biting midges *Culicoides* Latreille (Diptera: Ceratopogonidae) and allied genera. Bull. Entomol. Res. 43:421–67.

Kettle, D. S., R. H. Nash and B. A. Hopkins. 1956. Field tests with larvicides against *Culicoides impunctatus* Goetgh. in Scotland. Bull. Entomol. Res. 47:553–73.

Linley, J. R. and G. H. Adams. 1972. Ecology and behavior of immature *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae). Bull. Entomol. Res. 62:113–28.

Linley, J. R. and D. S. Kettle. 1964. A description of the larvae and pupae of *Culicoides furens* (Poey), and *Culicoides hoffmani* Fox (Diptera: Ceratopogonidae). Ann. and Mag. Natur. Hist. Ser. 13:7:129–49.

Williams, R. W. 1960. A new and simple method for the isolation of fresh-water invertebrates from soil samples. Ecology 41:573–4.

Wirth, W. W. 1952. The immature stages of two species of Florida salt marsh sandflies (Diptera: Heleidae). Fla. Entomol. 35:91–100.

RELATIONSHIP BETWEEN THE AMOUNT OF CO_2 AND THE COLLECTION OF TABANIDAE IN MALAISE TRAPS

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ABSTRACT. The attractiveness of different amounts of CO_2 for tabanids was compared based on collections in Malaise traps baited at rates of 0, 100, 1000, 2000, 3000, 4000, and 5000 ml/min. The collections of 10 of the 18 species taken were statistically analyzed. Analysis of variance based on the transformation of the data to log $(X + 1)$ was the most satisfactory of the 4 transformations studied. Regression analyses were made with 9 models; the most satisfactory model was log $(Y + 1) = a + bX + cX^2$. The resulting regression equations predict that rates of release of CO_2 above 4000 ml/min will reduce the number of flies collected and thus lead to erroneous interpretation of population dynamics. A black object used as an alternate bait did not attract some species but was the equivalent for other species to a rate of CO_2 of 100 ml/min.

INTRODUCTION. In a previous investigation (Roberts 1971) of the influence of CO_2 on tabanids collected in Malaise traps, the maximum level of the gas investigated was 2 liters/min. However, the data indicated that there was a linear response in numbers collected to amount of CO_2 released and that higher levels would increase the numbers collected. Subsequently, cattle were found capable of releasing as much as 3.5 liters/min of CO_2 (Roberts 1972). The present study was made to determine the effect on collections in Malaise traps of as much as 5 liters/min of CO_2.

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[2] Mention of a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the USDA.

MATERIALS AND METHODS. Eight new Malaise traps (Townes 1962) constructed of natural-color saran screen were placed on the shoulders of the roads surrounding the four sides of a 1.25 x 0.5 mile (2.01 x 0.8 km) rectangular area in the Delta Experimental Forest, Stoneville, Mississippi. Two traps were placed on each side, and there was a distance of at least 0.3 mile (0.48 km) between traps.

The CO_2 was released at the center pole of each Malaise trap about 3 feet above ground level from a 50-lb tank through a single-stage regulator and adjusted with a needle valve to the desired rate of flow with a Gilmont® compact flow meter. The gas was released at rates of 0, 100, 1000, 2000, 3000, 4000, and 5000 ml/min. In addition, the attractancy of a doughnut-shaped object constructed of four 6-inch stovepipe elbows painted with glossy black enamel was compared to that of CO_2.

This object was hung horizontally from the trap frame in the trap opening facing the road and was approximately 10 inches below the top of the opening. An 8 x 8 randomized Latin square design was used to assign each treatment to each trap site on each of the 8 collection periods. Each collection period was begun between 2:00 and 2:30 p.m. (Central Daylight Time) and terminated the following day between 10:00 and 10:30 a.m. Collections were made between July 1 and 16, 1970.

Analysis of variance of the data was made on X (number of flies collected) and the transformations: Log $(X+1)$; log $(X+5)$; $\sqrt{X+5}$; and $\sqrt{X+1}$.

The regression of flies collected on CO_2 levels was made for the following 9 models where Y = number of flies and X = rate of CO_2 in ml/min.

$$Y = a + bX$$
$$Y = a + bX + cX^2$$
$$Y = a + bX + cX^2 + dX^3$$
$$\sqrt{Y + 0.5} = a + bX$$
$$\sqrt{Y + 0.5} = a + bX + cX^2$$
$$\sqrt{Y + 0.5} = a + bX + cX^2 + dX^3$$
$$\text{Log}(Y + 1) = a + bX$$
$$\text{Log}(Y + 1) = a + bX + cX^2$$
$$\text{Log}(Y + 1) = a + bX + cX^2 + dX^3$$

RESULTS AND DISCUSSION. Eighteen species of Tabanidae were collected in the study. The collections of 10 of these species were statistically analyzed. The collection numbers of the 8 species omitted from analysis are included in Tables 1 and 2 under total tabanids. These were: *Chrysops callidus* Osten Sacken, *Leucotabanus annulatus* (Say), *Tabanus calens* L., *T. equalis* Hine, *T. mularis* Stone, *T. sulcifrons* Macquart, *T. venustus* Osten Sacken, and *T. wilsoni* Pechuman. The total number of flies collected by each treatment for each of the 10 species is presented in Table 1. Analyses based on the transformation of the data to log $(X+1)$ were the most satisfactory for stabilizing the irregular heteroscedasticity that was encountered in the collection data. When Duncan's multiple range test

Table 1. Total number of each species collected in Malaise traps baited with a black object or CO_2 (total of 8 collections).

Species	Black object	Rates of CO_2 released (ml/min)						
		0	100	1000	2000	3000	4000	5000
Chlorotabanus crepuscularis (Bequaert)	2 a	3 a[a]	15 b	106 c	193 cd	208 d	224 d	247 cd
Chrysops flavidus Wiedemann	97 a	95 a	258 b	1127 c	987 c	1508 c	1749 c	1153 c
Tabanus								
abdominalis F.	262 b	144 a	672 c	2233 d	4267 e	4094 e	4100 e	5101 e
americanus Forster	0 a	0 a	3 ab	9 bc	8 abc	13 c	28 c	17 c
atratus F.	5 ab	0 a	1 a	1 a	5 ab	11 b	9 b	12 b
cymatophorus Osten Sacken	4 ab	2 a	4 ab	10 abc	18 abc	18 bc	21 c	27 c
fuscicostatus Hine	2241 b	626 a	1953 b	3008 c	4310 cd	5210 d	5365 d	4078 cd
lineola F.	42 a	26 a	49 a	152 b	184 bc	203 c	224 c	231 c
proximus Walker	201 b	39 a	235 b	581 c	829 d	1079 d	977 d	912 d
subsimilis Bellardi	330 b	118 a	486 b	1019 c	1163 cd	1443 d	1418 d	1363 d
All tabanids	3194 b	1059 a	3704 b	8300 c	12006 cd	13852 d	14232 d	13208 d

[a] For each species, numbers followed by the same letter are not significantly different. Duncan's separation based on analyses of the transformation log $(X+1)$.

Table 2. Regression equations of the model log $(Y + 1) = a + bX + cX^2$ for the effect of CO_2 on collection numbers and R^2 % values for seven species of tabanids and the total number of tabanids collected.

Species	Regression equation [a]	R^2 %
Tabanus		
abdominalis	$\text{Log}(Y + 1) = 1.493 + (6.9 \times 10^{-4})X + (-9.9 \times 10^{-8})X^2$	50.5
fuscicostatus	$\text{Log}(Y + 1) = 2.065 + (3.9 \times 10^{-4})X + (-5.6 \times 10^{-8})X^2$	38.9
lineola	$\text{Log}(Y + 1) = 0.658 + (3.9 \times 10^{-4})X + (-5.6 \times 10^{-8})X^2$	50.2
proximus	$\text{Log}(Y + 1) = 1.032 + (6.5 \times 10^{-4})X + (-9.5 \times 10^{-8})X^2$	64.5
subsimilis	$\text{Log}(Y + 1) = 1.376 + (5.2 \times 10^{-4})X + (-7.4 \times 10^{-8})X^2$	60.2
Chrysops		
flavidus	$\text{Log}(Y + 1) = 1.249 + (5.2 \times 10^{-4})X + (-7.8 \times 10^{-8})X^2$	38.5
Chlorotabanus		
crepuscularis	$\text{Log}(Y + 1) = 0.257 + (6.5 \times 10^{-4})X + (-9.5 \times 10^{-8})X^2$	58.4
Total tabanids	$\text{Log}(Y + 1) = 2.354 + (5.2 \times 10^{-4})X + (-7.4 \times 10^{-8})X^2$	58.6

[a] All coefficients significant at 0.01 level or better. Y = No. of flies; X = CO_2 in ml/min.

was applied to the transformed data, the extensive overlapping between treatment means encountered with the untransformed data was greatly reduced.

The attraction of the different rates of CO_2 varied between species. Curves of the ratio obtained by dividing the numbers collected at each rate by the numbers collected at the zero rate against the amount of CO_2 released were generally irregular above 2000 ml/min on a species basis. However, when species were disregarded and the total tabanids were plotted against rates of CO_2, the curve was remarkably uniform (Fig. 1) indicating that the entire population of tabanids responded uniformly to the incremental increases in rates of CO_2.

Since CO_2 is obviously the tabanid host-finding mechanism, a comparison of the tabanid response to various rates of CO_2 to the release rates of CO_2 by cattle is applicable at this point. Lactating dairy cattle (Holstein) released CO_2 at the rate of 3.34 liters/min (Flatt et al., 1967) and heifer beef cattle (Herefords) released CO_2 at rates from 1.2 to 1.8 liters/min depending on whether the animals had a maintenance or a gaining ration (Roberts 1972). These rates coincide closely with the peak of the curve or with the transition point of the curve between the rapid rise in response to an increase in CO_2 and the plateau of response, respectively.

The attraction of tabanids to the black object varied on a species basis. For example, the numbers attracted to the black object and the control (unbaited trap) did not differ significantly for 6 species, but for 3 species the attraction of the black object was equal to that of CO_2 released at a rate of 100 ml/min. Also, traps baited with the black object collected significantly more *T. abdominalis* than the control traps but significantly less than traps baited with 100 ml/min with CO_2.

A significant t-value for all the coefficients was the primary basis for selecting a regression equation model. In those cases in which two or more models had all coefficient values significant, the one with the highest R^2 value was chosen. The most satisfactory model was log $(Y + 1) = a + bX + cX^2$. The regression equations for 7 species and total tabanids are presented in Table 2. The negative value of the X^2 term in the equation indicates that the flies responded in a manner such that large amounts of CO^2 ($>$ 4000 ml/min) would act as a repellent. This effect would be particularly important in trapping studies in which large amounts of CO_2 released within the trap would reduce collection numbers and thus lead

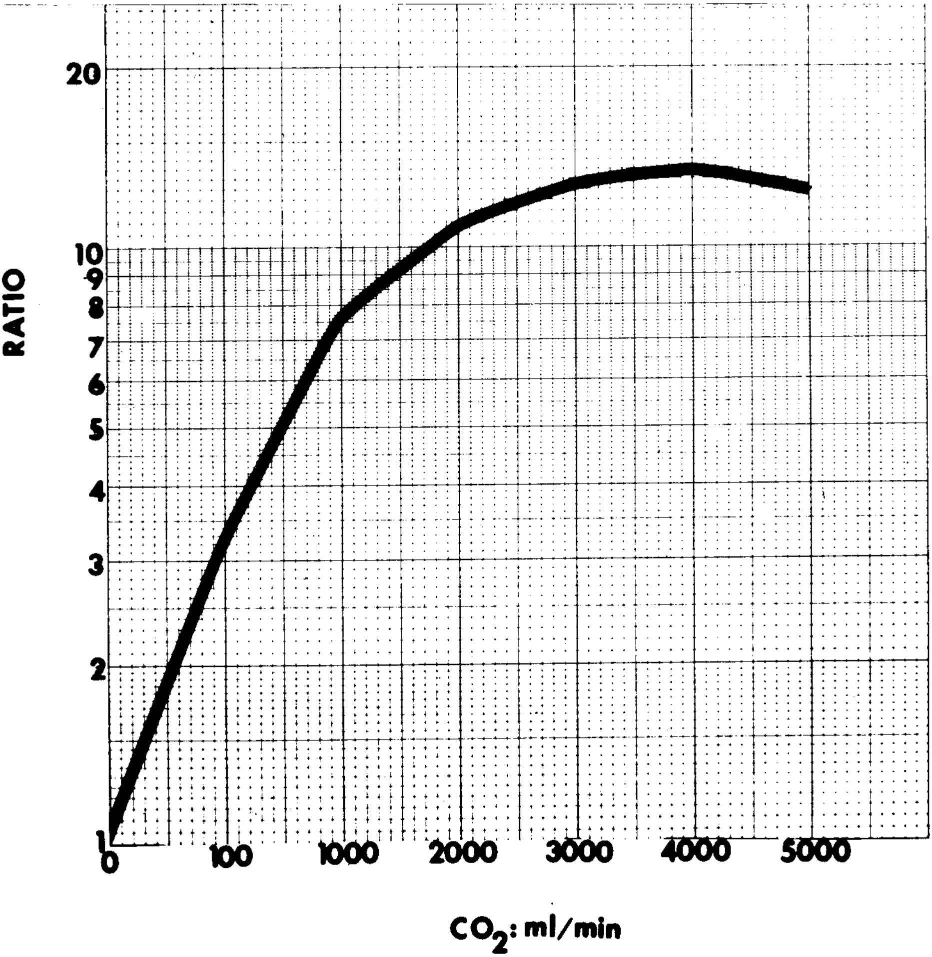

Fig. 1. Graph of the ratio: $\frac{\text{No. flies collected per rate of } CO_2}{\text{No. flies collected at zero } CO_2}$ plotted against rates of CO_2.

to the erroneous interpretation of populations.

When the equations were used to predict the number of flies that would be collected at each rate of CO_2, the Y values were considerably smaller than the corresponding (original) collection numbers though the curve generated from the Y-value was similar to that of the curve for the original data. However, the regression equations were based on the relationship between CO_2 and the number collected. Since the degree of CO_2 influence on capture is indicated by the magnitude of the R^2 value, other factors such as meteorological effects, population cycles, and those that affect site differences have an influence on the tabanid response and need to be investigated.

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References Cited

Flatt, W. P., P. W. Moe, L. A. Moore, N. W. Hooven, R. P. Lehmann, E. R Ørskov and R. W. Hemken. 1969. Energy utilization by high producing dairy cows. I. Experimental design, ration composition, digestibility data, and animal performance during energy balance trials. 4th Symposium on Energy Metabolism of Farm Animals, European Association of Animal Products. Oriel Press Ltd., New Castle on Tyne, England. Publ. 12, 522 p.

Roberts, R. H. 1971. Effect of amount of CO_2 on collection of Tabanidae in Malaise traps. Mosq. News 31:551–558.

Roberts, R. H. 1972. Relative attractiveness of CO_2 and a steer to Tabanidae, Culicidae, and *Stomoxys calcitrans* (L.). Mosq. News 32: 208–11.

Townes, H. 1962. Design for a Malaise trap. Proc. Entomol. Soc. Wash. 64:253–262.