PHOSPHORUS^{32} FOR MARKING TABANIDAE (DIPTERA) [1]

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INTRODUCTION

The introduction of low cost, reliable isotope detectors and an array of radioisotopes has encouraged the use of isotopes for marking animals in population studies. The availability of both detection equipment and isotope (phosphorus^{32}) at the Wildlife Research Station, Algonquin Park, Ontario, during the summer of 1966 made it possible to apply this technique to the local population of tabanids. The study was particularly directed to the development of an efficient method of marking tabanids in order to study their movements from a central release point. In addition, information was obtained on the rate of elimination of the isotope from the flies under laboratory conditions, methods of trapping and the response of these sylvatic flies to certain visual stimuli.

METHODS

Tabanids were caught in cubic cages (2 x 2 x 2 ft.) covered with white gauze on five sides; the cages were raised on stands 18 inches above the ground. The outlet from a cylinder of compressed carbon dioxide was situated beneath the cage and gas was released at a rate of either 175 cc./min. or 350 cc./min. This trapping procedure, utilizing carbon dioxide as an attractant, was similar to that described by Smith (1966). Some tabanids, primarily Chrysopinae, were caught in net sweeps about man.

Labelled flies were detected with a Nuclear-Chicago Model 8770 Educational Scaler equipped with a D33 Geiger-Mueller detector mounted in a No. 404 holder. The scaler was also equipped with a model 8420 Dual Timer.

Isotope in the form of $H_3P^{32}O_4$, was obtained from Atomic Energy of Canada Ltd. The stock isotope was diluted with a Sorenson phosphate buffer (pH 7) to the approximate level of 1 mc/50 cc.

RESULTS

TECHNIQUES

TRAPPING TABANIDAE. A standard trap cage, based on that described by Fallis and Smith (1964) and Smith (1966), was used initially. Our own observations, together with those reported in the literature (Thorsteinson, 1958; Bracken, Hanec and Thorsteinson, 1962; Thorsteinson, Bracken and Hanec, 1965; Bracken and Thorsteinson, 1965; Thorsteinson, Bracken and Tostowaryk, 1966) indicated that tabanids are attracted to colours and surfaces of low reflectivity. An experimental cage was designed in which a strip of black paper, 6 inches wide, was attached exteriorly, around the bottom of a gauze cage. This cage and a cage of identical dimensions but lacking the black paper rim, were operated within 8 feet of each other (Fig. 1, site 5). In another experiment, black or white triangles of paper (14 inches high and 5 inches on the base) were suspended beneath the open bottoms of trap cages placed on stands. These two cages were operated within 8 feet of each other at site 3 (Fig. 1). Carbon dioxide, at approximately equal rates (*circa* 175 cc./min.) was released at the base of each cage at each site. The flies were collected daily during the period 0800–2000 hours. The figures in Tables 1 and 2 represent the number of flies obtained during each of 18 daily collections.

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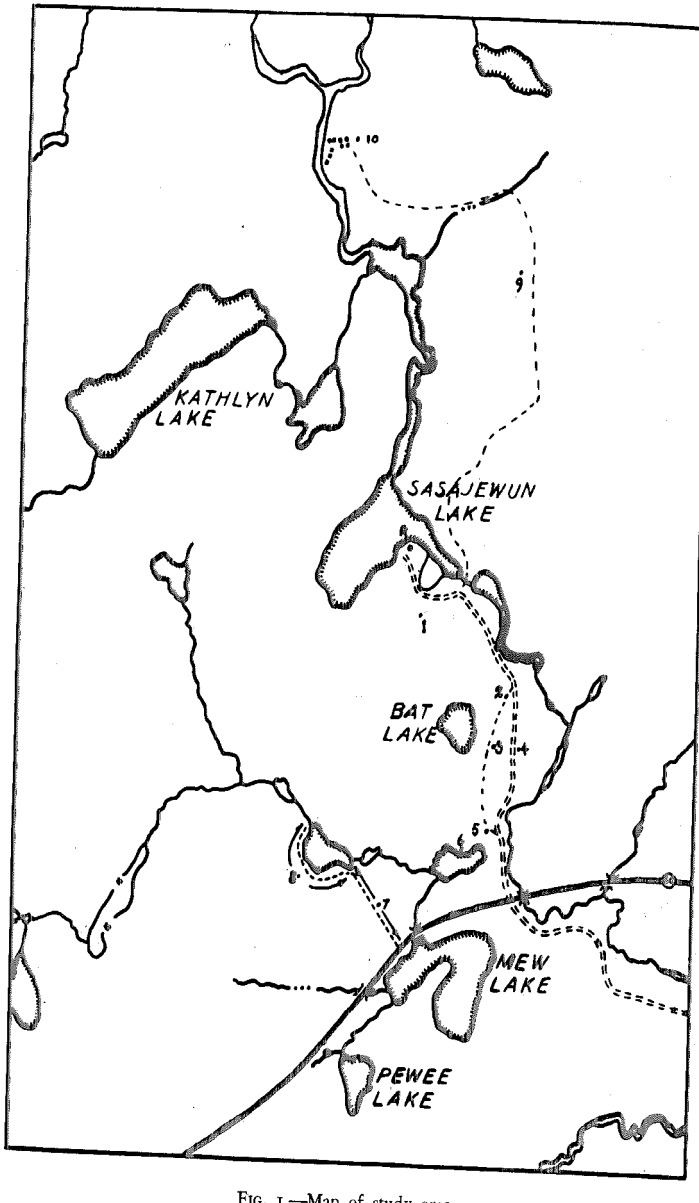

FIG. 1.—Map of study area.

In eighteen tests (Tables 1 and 2), the white cages contained more flies on only three occasions, with a total of 663 flies captured. The dark cage and the cage with the black triangle had more flies on fifteen occasions, with a total of 867 tabanids captured. The results suggest that the "black-equipped" cages were slightly more attractive than the corresponding white cages.

The results (Table 1) suggest that the cage with black paper was almost equally attractive to both groups but that nearly twice as many of the Chrysopinae entered the cage without the black paper. The two sets of data, considered together, suggest that the Tabaninae "prefer" the cage equipped with black paper strips. Thus, neither experimental arrangement could be used to determine the population composition accurately. However, the results in Table 2 indicate that the two groups of tabanids were caught in similar proportions in both cages, although the cage with the black triangle caught almost twice as many flies. As a technique,

TABLE 1.—Comparison of the number of flies caught in a cage with a 6 inch strip of black paper around the bottom with those caught in a similar cage without black paper. Carbon dioxide flow in each cage approximately equal.

	Cage with black paper			Cage without black paper		
Expt.	Tabaninae	Chrysopinae	Total	Tabaninae	Chrysopinae	Total
1	110	145	255	103	175	278
2	45	8	53	23	71	94
3	14	32	46	23	7	30
4	26	11	37	3	6	9
5	6	13	19	0	2	2
6	35	11	46	20	23	43
7	18	18	36	9	20	29
8	35	17	52	3	8	11
9	12	16	28	1	4	5
10	10	9	19	5	16	21
Total	311	280	591	190	332	522
Mean	31.1	28.0	59.1	19.0	33.2	52.2
% of total	52.6	47.4		36.4	63.6	

TABLE 2.—Comparison of the number of flies caught in cages provided with black and white triangles suspended beneath the cages. Carbon dioxide was released at each cage in approximately equal quantities.

	Cage with black triangle			Cage with white triangle		
Expt.	Tabaninae	Chrysopinae	Total	Tabaninae	Chrysopinae	Total
1	36	29	65	17	26	43
2	20	7	27	3	3	6
3	3	1	4	2	0	2
4	24	22	46	18	11	29
5	19	18	37	7	12	19
6	9	16	25	5	8	13
7	26	30	56	16	7	23
8	10	6	16	4	2	6
Total	147	129	276	72	69	141
Mean	18.4	16.1	34.5	9.0	8.6	17.6
% of total	53.3	46.7		51.1	48.9	

therefore, this second method would seem to be preferable to the first for estimating the population composition, but the first would seem superior for catching greater numbers of tabanids, especially when large numbers are needed in mark-release experiments.

Additional experiments indicated that the height of the cage-trap above the ground is also an important factor in determining the relative composition of the collection. In six tests, mean captures of Chrysopinae in traps at 14 inches and 34 inches above the ground were equal (8.8 and 8.5 respectively). Mean captures of Tabaninae were 10.8 in traps at 14 inches and only 2.0 in traps at 34 inches, indicating a greater efficiency of the lower traps for this group. In other experiments no tabanids were captured in traps placed with the open bottom 6 feet above the ground.

Flies captured in gauze-covered cages were usually in excellent condition with little evidence of wing damage. This was a decided asset when flies were being captured for isotope marking and subsequent release.

ISOTOPE LABELLING. Tabanids caught in cage traps or by net sweeps about man were placed in 12-inch cubic cages with one side of plexiglass, one of nylon gauze and the remaining sides of wood. The isotope, in appropriate dilution (usually 1 mc./50 cc. buffered water) was sprayed through the gauze with an atomizer. The flies readily drank the free water droplets on the interior of the cage; all flies in the cage could be marked within 30 minutes, particularly if held in a trap cage all day. The Tabaninae usually picked up more of the isotope, probably by ingestion (an average of 50,000 d.p.m. for 38 flies) than the Chrysopinae (average of 37,000 d.p.m. for 27 flies).

In order to reduce damage to the wings resulting from flight activity within a confined space, it was found convenient to keep the flies in total darkness before and after spraying with isotope. In addition, the Chrysopinae tended to die rather rapidly if kept in cages too long. *Chrysops* spp. maintained in covered cages overnight and provided with sugar and water suffered mortalities of from 40 percent to 70 percent and approximately half of the survivors would be flightless. Survival was increased when the flies were kept in captivity for as short a period as possible (30–45 minutes). All labelled flies, regardless of the site of capture, were released at the Wildlife Research Station (Point R, Fig. 1).

To determine the efficiency of the labelling technique it was necessary to ascertain whether the labelled flies retained the isotope for sufficient periods of time or whether they excreted it rapidly. The rate of loss of the isotope was determined for captive tabanids. On each day the flies were anaesthetized with diethyl ether and the isotope level determined. Five tests were carried out on both the Tabaninae and the Chrysopinae. Most of the Tabaninae involved were *Hybomitra epistates* (Osten-Sacken), H. *affinis* (Kirby), including the subspecies *H. affinis aurilimba* (Stone), *H. typhus* (Whitney) and *H. trispila sodalis* (Williston) with fewer numbers of the other species available (Table 4). The Chrysopinae comprised mostly *C. excitans* Walker, *C. frigidus* Osten-Sacken, *C. montanus* Osten-Sacken, *C. indus* Osten-Sacken, *C. shermani* Hine and *C. vittatus* Wiedemann with a few indivduals of *C. mitis* Osten-Sacken, *C. carbonarius* Walker and *C. univittatus* Macquart.

The results were variable throughout the five tests; the Chrysopinae showed the least variability while the results for the Tabaninae were markedly different. Fig. 2 was chosen as the experimental results with the worst fit to a theoretical decay curve, while Fig. 3 was chosen as the best fit. The sharp drop in levels of isotope (Fig. 2) remained unexplained. Following the sharp drop, the rate of loss is fairly uniform and similar to that expected from natural decay. As both groups of flies showed a similar pattern in this test, it is possible that experimental error

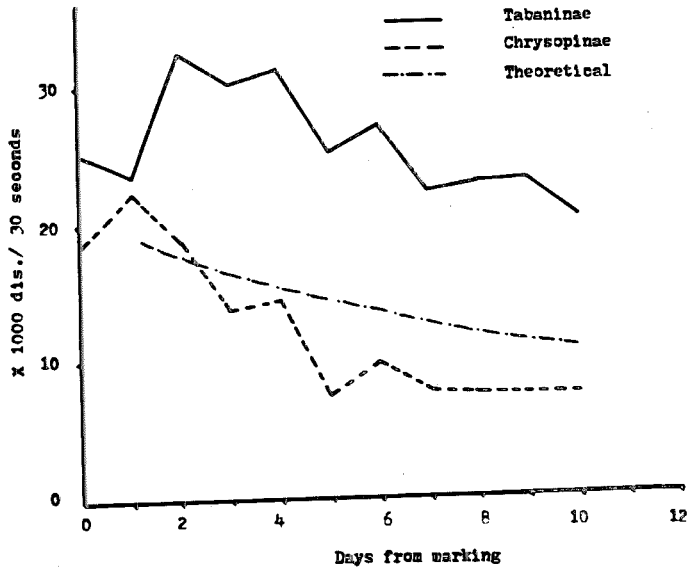

FIG. 2.—Rate of elimination of isotope by caged tabanids.

caused the sharp drop illustrated. Pooled results for all species on comparable days following marking gave a curve (Fig. 4) which is in good agreement with the theoretical decay curve.

It is possible that the variability seen in the individual experiments is a result of small sample size. An analysis of the data for the individual species did not indicate any notable variation from the pattern for that experiment. Apparently the flies do not specifically excrete the isotope and the loss of isotope is due basically to natural decay. Flies remained marked (Fig. 2) at a high level for at least 20 days following labelling. The level of the label at the end of this period was high enough to permit easy recognition of marked individuals. Under field conditions, when egg-laying occurs, however, the flies could be expected to lose isotope more rapidly if the isotope is incorporated into the eggs as described for *Aedes aegypti* (L.) (Bennett, 1965).

Control groups of unlabelled tabanids were maintained to determine if the presence of isotope in the fly was harmful, as indicated by increased mortailities. These control tabanids were kept under the same conditions as the labelled flies and were subjected to a daily anaesthetization as for the labelled flies. The anaesthesia itself resulted in some mortality. However, no difference in mortality of the two groups was noted and it was assumed that the isotope, at the levels used, did not shorten the life of the individual fly.

Distribution of the isotope in the flies was determined by initially noting the level of the isotope in the intact fly (*C. excitans*). The fly was then dissected and isotope levels (corrected for background) for the head, thorax, abdomen, and legs and wings were determined. The total (Table 3) radiation measured in the parts was 0.2 percent less than the total recorded for the intact fly. Approximately one-half of the isotope occurred in the abdomen, with about one-quarter in the thorax and the remainder in the head, legs, and wings. The pattern of isotope distribution was similar to that found in *Simu-*

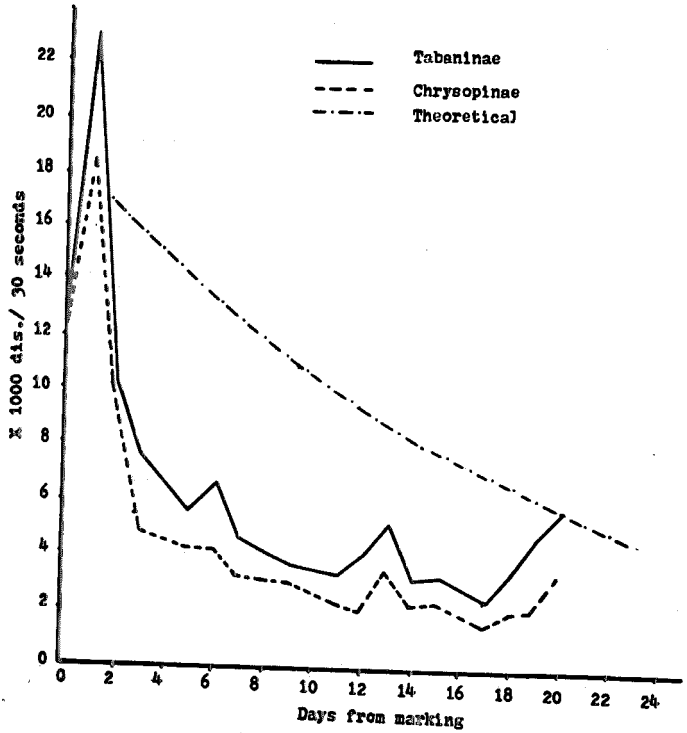

FIG. 3.—Rate of elimination of isotope by caged tabanids.

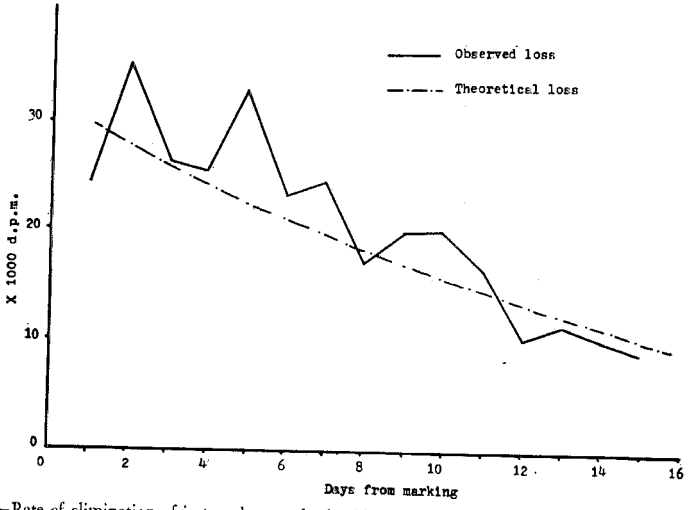

FIG. 4.—Rate of elimination of isotope by caged tabanids. Pooled results for all tabanids on comparable days following marking.

TABLE 3.—Distribuiton of P^{32} in six specimens of *Chrysops excitans*, approximately seven days following marking. Figures in percent columns indicate percent of isotope in that portion of the fly, based on the total isotope from all parts.

Quantity of isotope in intact fly (d.p.m.)	Head %	Thorax %	Abdomen %	Legs & wings %	Total d.p.m.
6734	8.7	22.9	46.6	21.9	6957
8719	8.4	19.8	49.9	21.9	7859
6639	10.2	25.9	52.3	11.6	6224
7661	10.4	35.2	45.8	8.6	7331
19400	7.8	29.3	52.9	9.9	19902
12633	9.4	24.9	52.1	13.6	12038
Means	9.2	26.3	49.9	14.6	

lium euryadminiculum Davies (Bennett, unpublished observations), differing in that there was comparatively more isotope in the legs and wings and less in the head of *C. excitans*.

FIELD STUDIES

RELEASE-RECOVERY. Approximately 800 Tabaninae and 2,220 Chrysopinae were labelled and released between June 23 and July 11, 1966. A total of 3,093 Tabaninae and 5,293 Chrysopinae were captured and the sample examined for labelled flies. One hundred eight horse flies, representing 3.5 percent of the Tabaninae captured and 13.5 percent of the Tabaninae marked and released, were labelled; 99 deer flies, representing 1.9 percent of the Chrysopinae captured and 4.5 percent of the Chrysopinae marked and released, were labelled. The tabanid population was sampled for labelled flies from June 25 through August 5. The species composition of a sample of the flies marked and released as well as the species composition of the labelled flies recaptured is given in Table 4. It was impractical to identify to species every specimen marked and released. Generally speaking, the species of marked flies recaptured were in the same proportion as those marked and released. *H. affinis, H. epistates, C. excitans* and *C. montanus* were among the more common species marked and released and represented the largest proportion (67.1 percent) of the flies recaptured.

An estimate of the density of the tabanid population in the study area was made by applying the formula of Bailey (1951) to the mark-recapture data; 3,020 flies were labelled and released and of 8,386 flies recaptured, 207 were labelled. Using these figures, the population of tabanids was estimated as 120,000 in the area under study during the 6-week period. Assuming that the area under study was a rectangle 2 miles by ¾ mile (Fig. 1) or 960 acres, the average fly density is approximately 125 flies per acre. Since little work was carried out in the northern portion of the area, it would be more accurate to estimate the area under study as ¾ square mile and a fly density of approximately 250 per acre (Table 5, Fig. 1), over the 6-week period.

DISTRIBUTION OF MARKED FLIES. All labelled flies were released at point R (Fig. 1). Sites 1–6 (Fig. 1) were static trapping sites at which cages baited with carbon dioxide were run from 10 to 12 hours daily over a minimum 4-week period. Most traps were operated for 6 weeks. Sites 7–10 represent areas rather than single points, in which net sweeps around man were made. Sites 7 and 8 had six such collections made on 6 different days. Sites 9 and 10 had a single collection. The number of flies examined and the percentage of marked individuals in the samples are given in Table 5. Labelled Tabaninae were recovered as far as one mile from the site of release. Labelled Chrysopinae were recovered as

TABLE 4.—Species composition of sample of flies marked and released, and of the labelled flies recaptured.

Species	In sample		Recaptured	
	No.	%	No.	%
Chrysops spp.				
carbonarius	8	1.2	1	1.0
cincticornis Walker	28	4.1	4	4.0
cuclux Whitney	20	3.0	3	3.0
excitans	232	34.3	39	39.4
frigidus	98	14.5	8	8.1
indus	53	7.8	1	1.0
lateralis Wiedemann	13	1.9	7	7.1
mitis	24	3.6	2	2.0
montanus	80	11.8	21	21.2
niger Macquart	8	1.2	3	3.0
shermani	79	11.7	7	7.1
univittatus	6	0.9	3	3.0
venus Philip	2	0.3	0	0.0
vittatus	25	3.7	0	0.0
TOTAL	676	100.0	99	99.9
Hybomitra spp.				
affinis	77	22.1	32	29.6
arpadi (Szilady)	5	1.4	4	3.7
criddlei (Brooks)	5	1.4	3	2.8
epistates	198	56.7	47	43.5
illota (Osten-Sacken)	5	1.4	2	1.9
lasiophthalma (Macquart)	1	0.3	1	0.9
metabola (McDunnough)	6	1.7	1	0.9
nuda (McDunnough)	4	1.1	0	0.0
trepida (McDunnough)	8	2.3	1	0.9
trispila sodalis	15	4.3	8	7.4
typhus	19	5.4	4	3.7
zonalis (Kirby)	1	0.3	1	0.9
undet.	4	1.1	4	3.7
Tabanus sp.				
marginalis Fabricius	1	0.3	0	0.0
TOTAL	349	99.8	108	99.9

TABLE 5.—Distribution of marked tabanids after release from a central marking point (Fig. 1).

Site no.	Distance from point of release (miles)	Tabaninae			Chrysopinae		
		Total examined	No. marked	% marked	Total examined	No. marked	% marked
1	1/4	964	20	2.1	690	11	1.6
2	3/8	158	2	1.3	149	0	0.0
3	1/2	460	48	10.4	568	13	2.3
4	1/2	214	7	3.3	348	5	1.4
5	3/4	794	25	3.1	1019	8	0.8
6	7/8	242	5	2.1	171	6	3.5
7	7/8	26	2	7.7	848	13	1.5
8	5/4	13	0	0.0	660	8	1.2
9	4/4	11	1	9.1	84	4	4.8
10	5/4	4	0	0.0	59	0	0.0

far as 1¼ miles from point R. Trapping or netting was not carried out at more remote points.

It was anticipated that the proportion of marked flies would decrease with the distance from the point of release. Presumably, therefore, the proportion of marked to unmarked flies ought to have been greatest at site 1 (Fig. 1). Not only was this site closest to the point of release but also a number of captive moose and deer were located at site 1 which, presumably, might have produced local aggregations of tabanids. However, the number of flies recaptured at site 1 was quite low. The proportion of marked flies recaptured at the other sites was quite variable (possibly because in some instances the samples were small) but at one site, site 3, 10.5 percent of the flies examined were labelled. Site 3 was located on an old road running through a sand plain in a birch-aspen-conifer forest. The sides of the road were somewhat overgrown with shrubbery (primarily *Corylus* sp.) and the site itself was partially enclosed by overhead trees. It was situated about 150 yards from a small lake. No penned animals were in the vicinity. Sites 2 and 5 were located along the same road, these sites differing from site 3 primarily in that the forest was more open. Why site 3 should have been so favourable for marked flies is not known. Similar 'microhabitat' preferences have been demonstrated for other biting Diptera as well as for the Tabanidae (Smith, 1966).

OBSERVATIONS OF RESPONSE TO VISUAL STIMULI. Response of tabanids (mostly *Chrysops* spp.) to visual stimuli was noted during the course of some studies on the black fly *Simulium venustum* Say. In these studies, cylinders 50 inches long by 9 inches in diameter were coated with "Tanglefoot" and placed in either vertical or horizontal positions. All cylinders were in sight of each other, approximately 10 to 15 feet apart. A flow of carbon dioxide (approximately 200 cc/min.) was present at either the end or the mid-point of some of the cylinders. Of 185 tabanids captured on the cylinders, 169 were *Chrysops*. In the same area (Fig. 1, site 1) a total of 964 Tabaninae and 690 Chrysopinae were taken in Manitoba Fly-traps (Thorsteinson, Bracken and Hanec, 1964) or modified versions of these traps. In 52 tests, all 185 tabanids were taken on cylinders equipped with a carbon dioxide outlet; no flies were taken on 7 control cylinders operated without carbon dioxide. These results confirm the attraction of tabanids to carbon dioxide first demonstrated by Smith (1966) and Wilson, Tugwell and Burns (1966).

The response of flies to vertical and horizontal cylinders supplied with carbon dioxide is shown in Table 6. Quite clearly, the tabanids responded to horizontally placed cylinders rather than the vertical, and responded mainly to those cylinders closest to the ground. In four additional experiments carried out simultaneously, using cylinders placed 36 inches and 12 inches from the ground, 13 tabanids (average of 3.3 flies/test) were taken at 36 inches and 83 flies (average of 20.8 flies/test) were taken at 12 inches from the ground. The distribution of flies as noted on cylinders 12 inches from the ground

TABLE 6.—Captures of Tabanidae on vertical or horizontal cylinders provided with carbon dioxide. Cylinders were coated with "Tanglefoot." Experiments were carried out at site 1 (Fig. 1). Figures in inches indicate height of the top-most point of the cylinder from the ground.

Position	Number of tests	Number of flies	Mean number test
Vertical (58")	41	4	0.1
Horizontal (72")	4	1	0.25
Horizontal (36")	11	43	3.9
Horizontal (12")	28	137	4.9

was 66 flies on the topmost portion of the cylinder (at random along the length) and 16 flies on the bottom and lateral portions of the horizontal cylinder.

DISCUSSION

The results of this brief study have shown that marking a population of tabanids with isotope is feasible and that the return of marked flies is sufficiently high to obtain meaningful results.

The rate and mode of P^{32} elimination and its distribution in the tabanids should be studied in detail; eggs laid by labelled flies should be measured for their isotope content. Similar studies should also be carried out using isotopes such as calcium and sulphur. Such a technique might present a new and profitable approach to the study of some of the physiological mechanisms of tabanids.

The brief behavioural studies reported here augment the observations on the behaviour of tabanids as described by Thorsteinson and his students and cited above. These authors reported that visual stimuli play an important role in the behaviour patterns of tabanids and the efficient Manitoba Fly Trap is predicated on this thesis. This trap is of considerable value in cleared or agricultural areas in which there is unobstructed visibility for considerable distances.

In Algonquin Park, however, a different situation obtains. For the most part this region is densely forested and the majority of species of tabanids are sylvatic (Smith, 1966). The potential for using vision is severely restricted in such habitats and cage traps operated without carbon dioxide as an attractant were unsuccessful in capturing tabanids. In the response of the tabanids to the silhouettes, although these may not have been ideal, it was clear that, in the absence of carbon dioxide, the cylinders did not catch tabanids, although all cylinders were visible from any one point. It would appear, therefore, that the sylvatic tabanids are dependent on stimuli in addition to vision for the location of the host. This does not imply that sight may not be the dominant factor at close range, but that vision from a distance is not possible in these areas except within limited locales such as provided by bogs, lakes and ponds.

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