

EXPERIMENTS AND OBSERVATIONS ON THE
NEAR-HOST ORIENTATION AND LANDING BEHAVIOR
OF SIMULIIDAE (DIPTERA)

CENTRE FOR NEWFOUNDLAND STUDIES

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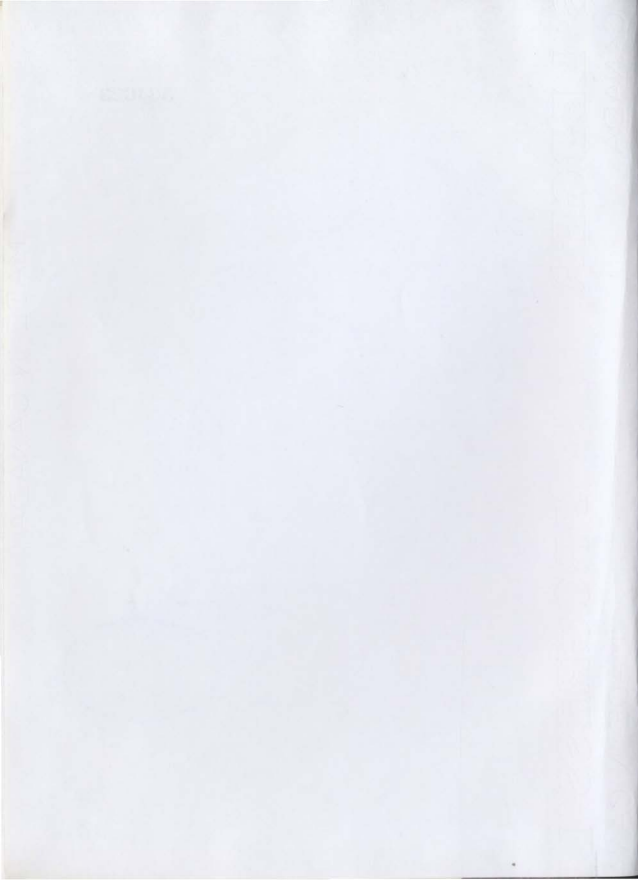
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EXPERIMENTS AND OBSERVATIONS
ON THE NEAR-HOST ORIENTATION
AND LANDING BEHAVIOR OF
SIMULIIDAE (DIPTERA)

by



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ABSTRACT

Records of the seasonal succession and seasonal range of several species of adult female mammalophilic Simuliidae of insular Newfoundland are presented.

Collections of mammalophilic Simuliidae on colored-shaped two-dimensional silhouettes indicated that various species orient and land on certain 'preferred' radiation compositions. The *Prosimulium mixtum* complex and *Cnephia mutata* were attracted consistently to black silhouettes; the *Simulium venustum* complex were attracted to silhouettes composed of 'blue' radiations while *Simulium vittatum* were orienting and landing on both red and black silhouettes. Black, 'blue' and red radiation compositions *per ordinem* were most attractive while yellow was the least attractive for all species, which indicated that radiation compositions reflecting the least amount of light were the most attractive, but, depending on the species composition of the simuliid population, this generalization tended to become obscure.

Responses of simuliids to simple geometric shapes indicated individual contours were equally attractive while the various 'points of convergence' of the silhouette shapes attracted large numbers.

Collections of flies orienting upwind to CO₂ obtained from a 'grid' of silhouettes indicated a decrease in the number of flies toward the row of silhouettes located downwind from the CO₂ point of emission. Depending on the individual silhouette 'colors' the flies were orienting to the CO₂ from as much as ten meters downwind.

Simuliids landed on black, disc-shaped silhouettes of different areas in proportion to the surface area exposed.

The responses of Simuliidae to 'attractive' and 'non-attractive' silhouettes and controlled release of gaseous CO₂ indicated a marked ability of simuliids to visually discriminate the targets largely on the basis of their individual radiation compositions and independent of the concentration of CO₂ or olfactory mechanisms. Discrimination coefficients indicated that distances approximating four meters downwind from the CO₂ source would appear to be a limiting distance for the simuliids' effective use of vision.

Long-range, medium-range and near-orientation mechanisms are presented for the Simuliidae in terms of the sensory make-up through which they are mediated.

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Perhaps the most valuable result
of all education is the ability
to make yourself do the thing
you have to do, when it ought to
be done, whether you like it or
not; it is the first lesson that
ought to be learned; and however
early a man's training begins, it
is probably the last lesson that
he learns thoroughly.

Thomas Henry Huxley
Technical Education [1877]

TABLE OF CONTENTS

LIST OF TABLES	iii
LIST OF FIGURES	iv
ACKNOWLEDGEMENTS	vi
INTRODUCTION	1
HISTORICAL SUMMARY	4
I Olfactory	5
II Hygroscopic	11
III Thermal	14
IV Visual	16
V Mechanical	21
VI Sound	21
VII Miscellaneous	22
MATERIALS AND METHODS	
A Description of the general area	23
B General collecting methods	24
C Specific collecting methods	29
I. Influence of colour and form	29
II. Influence of area	34
III. Influence of colors and carbon dioxide emission rates	34
RESULTS	36
Experimental Studies	40
Experiments and Observations in 1970	41
The influence of color and shape	41
Experiments and Observations in 1971	45

I.	The influence of color and shape	45
II.	The influence if silhouette size and surface area	49
III.	The influence of color and carbon dioxide emission rates	51
DISCUSSION		58
	The role of various radiation compositions	65
	The role of silhouette shape	78
	The role of silhouette size and surface area	79
	The importance of vision and olfaction	81
SUMMARY		95
BIBLIOGRAPHY		98
APPENDIX I		137
	Table of Variance	138

LIST OF TABLES

Table I	43
Table II	48
Table III	50
Table IV	52
Table V	53
Table VI	54
Table VII	55

LIST OF FIGURES

		following page
Fig. 1	Aerial view of Pickavance Creek	112
Fig. 2	Simple two-dimensional geometric forms, variously colored	113 114
Fig. 3	Rotating display platform of the 1971 design	115
Fig. 4	Saran wrap technique for protection of exposed silhouettes	116
Fig. 5	Meteorological instruments	117
Fig. 6	Exposure grid for silhouettes	118
Fig. 7	Horizontal display of stimulus cards	119
Fig. 8	Projection booth	120
Fig. 9	Testing combinations of Munsell Standard Colors	121
Fig. 10	Disc-shaped stimuli of varying areas	122
Fig. 11	Testing ability of Simuliidae to discriminate between stimuli with varying CO ₂ emissions	123 124
Fig. 12	Testing ability of Simuliidae to discriminate between stimuli separated by varying distances	125 126
Fig. 13	Seasonal succession of Simuliidae	127
Fig. 14	Landing responses of Simuliidae to color, 1970	128
Fig. 15	Landing responses of Simuliidae to shape, 1970	129
Fig. 16	Landing responses of Simuliidae to luminous reflectance, 1970 and 1971	130
Fig. 17	Landing responses of Simuliidae as a function of distance from CO ₂ source	131
Fig. 18	Landing responses of Simuliidae to color, 1971	132
Fig. 19	Landing responses of Simuliidae to shape, 1971	133

Fig. 20	Landing responses of Simuliidae to disc-shaped silhouettes of varying area	134
Fig. 21	The variation in intensity with wavelength for each silhouette pigment	135
Fig. 22	The theoretical downwind distances involved in near-orientation of mammalophilic Simuliidae	136

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INTRODUCTION

The importance of haematophagous Diptera in medical and veterinary entomology has been clearly defined since Ross (Russell *et al.*, 1963) in 1898 demonstrated the role of mosquitoes in the transmission of *Plasmodium* spp. Since that time, a considerable volume of literature has been developed relating the activity of biting flies to transmission of disease. Clearly, the medical importance of biting Diptera as vectors of disease is most pronounced in the tropical and sub-tropical areas, declining in importance as one approaches the temperate zones. In Canada, there is relatively little human disease transmission by biting flies, their medical importance lying primarily in the mechanical effects attendant on their mode of feeding (Davies and Peterson, 1956; Davies *et al.*, 1962; Fredeen, 1969). The Simuliidae of Canada are of particular importance in this field, and through the sheer density of the populations in the short northern summer, present a serious obstacle to the development of Canada's northlands. In addition to this activity, simuliids in Canada are vectors of disease of wildlife (Shewell, 1955; Anderson, 1956, 1961; Anderson *et al.*, 1962; Fallis and Bennett, 1966; Khan and Fallis, 1968), the importance of which cannot as yet be accurately assessed.

Although the medical importance of biting flies is fairly well known, the factors governing the biting activity and feeding behavior are best known for the Culicidae but much less understood for the Simuliidae. It has been assumed in the past that responses of the Simuliidae are similar to those of the Culicidae, but there has been

little experimental verification. The reasons for this are simple: Adult simuliids are exophagous and practical breeding in the laboratory is still an elusive goal. Consequently, simuliids must be studied in the field in situations where all the standard parameters vary continuously. Only recently have studies investigated the host-finding and attacking program of simuliids in the field (Smith, 1966; Golini, 1970).

The total feeding behavior of simuliids has been divided (Smith, 1966) and subdivided (Golini, 1970) into successive stages of behavior patterns, operative when seeking a host. The nature of this theoretical hierarchy is summarized as follows:

- PHASE I. Appetitive Behavior
 - Stage 1. Habitat selection
 - Stage 2. Upwind orientation
 - Stage 3. Near orientation
 - Stage 4. Landing behavior
 - Stage 5. Crawling and burrowing
- PHASE II. Consummatory Reaction
 - Stage 6. Probing and feeding
- PHASE III. Cessation of Feeding
- PHASE IV. Physiological State of Rest

The descriptions and discussion of experiments conducted under the present study will consider the first four hierarchal stages involved in Phase I above. It is assumed here that stages 1 and 2 are operative and factors influencing the successive two stages viz. 3 and

4 are investigated. Since stage 4 is mediated by visual stimuli and acknowledged by Smith (1966) as the termination of the 'in-flight' orientation, the landing behavior is considered here as the final behavioral element contingent on near orientation.

HISTORICAL SUMMARY

Literature pertaining to the activity of haematophagous Diptera, their relationship to vector-borne disease and predominant role as nuisance arthropods of both man and animal alike, are voluminous. Dating back to his explorations in Canada, 1599-1613, Samuel de Champlain's accounts probably represent one of the earliest reports of mosquitoes attacking man in North America which, incidentally, may have included black-flies (initially, Simuliidae were not differentiated from Culicidae, Davies *et al.*, 1962). Pioneers in Canada endured similar hardships during the ensuing years. By the early nineteen-fifties, a great deal of information had been published on the use of repellants against biting and especially disease-transmitting insects (Hocking, 1952). However, there was little understanding of the factors which attract these insects to their host and those which stimulate them to feed.

Following World War II and the experience gained therein on the importance of controlling vectors of insect-borne diseases, interest and research on these factors proliferated enormously. Much of the study was directed toward tropical insect-borne disease and concentrated largely on factors influencing mosquitoes. This vast literature collection of the past 25 years has thus ignored the Simuliidae in the main. The following historical account of the development of concepts leading to an explanation of the hierarchy of host-seeking-feeding behaviour is perforce, largely drawn from the work on the Culicidae. The feeding behaviour of the Simuliidae has, in the past, been interpreted on the basis of these data where specific evidence for the

Simuliidae is lacking. For easier comprehension, various factors involved in the host-seeking-feeding hierarchy of responses are treated separately.

I. OLFACTORY

i.) Carbon dioxide

Carbon dioxide was first reported by Rudolfs (1922) to be a mosquito activator and has been considered ever since to be one of a complex of factors attracting these insects to man, as well as an all-purpose, non-specific stimulant for many kinds of arthropods. It has been referred to as one of the most controversial elements in the whole history of host attractancy (Hocking, 1971). Some authors have concluded that it attracts while others have surmised repelling properties that appear inherent with the chemical. In most cases where carbon dioxide has been ultimately described as a repellent, it is probable that methods employed to release the gas were, initially, not carefully considered. For instance, CO₂ in the form of dry-ice may, in fact, cause chilling or subsequent anaesthesia of the insects coming in contact with it (Hocking, 1971). Other experiments have utilized the gas in various sized cages, and in such confined spaces there may be an accumulation of gas that could affect the caged insects in ways differing from those under field conditions. Thus, experiments using these and similar techniques may be suspect.

Dalrat (1950) treated simuliids with CO₂ gas prior to their feeding on a host, and successfully induced them to oviposit in captivity. Reeves (1951) was able to enhance collections of mosquitoes in his stable traps with the addition of CO₂, and suggested a direct

interrelationship with a mosquito's host seeking behaviour (Reeves, 1953). Important empirical data contributed by Brown (1951a, 1951b, 1952), Thompson and Brown (1955) presented further evidence of female mosquito chemotropisms and the attractiveness of vapours of CO_2 . Through morphological studies on types of chemo-receptors on the antennae of *Aedes aegypti* mosquitoes, Willis and Roth (1952) noted their attractiveness to several streams of air and CO_2 mixtures. Van Thiel and Laarman (1954) and Laarman (1958) found that CO_2 was a very strong attractive stimulus but that it formed part of a complex with other chemical 'odours' emitted by the host. Again, Laarman (1955) concluded that the essential role of CO_2 in orientation of *Anopheles maculipennis atroparvus* was that of an activating stimulus. By removing dissolved CO_2 from beef blood with BaCl_2 , Burgess and Brown (1957) could dissipate its attractiveness to *A. aegypti* but that upon passage of CO_2 over this blood its attractiveness was restored. Associating wind orientation and a mosquito's position in space with that of Johnston's Organ, Bässler (1958) maintained that near-orientation to a manikin was affected through CO_2 stimulation even with this organ removed. Upon viewing the probing behaviour of *A. aegypti*, Burgess (1959) found that the mosquito response depended either on the presence of a host or upon an increase in the amount of artificial CO_2 released in the vicinity. Further studies have shown conclusively that certain stimuli such as warm, moist air currents and CO_2 are necessary for mosquitoes to locate a host (Kellogg and Wright, 1962a, 1962b; Wright, 1962; Wright and Kellogg, 1962a, 1962b).

In search of 'other factors' besides CO_2 in the attraction of

mosquitoes to a host, Lipsitz and Brown (1964) tested 26 amino acids and found lysine was the most attractive amino acid in human blood but that the degree of mosquito receptivity was directly proportional to the amount of uncombined CO_2 it adsorbed. Wright *et al.* (1964 (1965)) calculated a mean biting rate for *A. aegypti* based on the number of each type of setae described on the antennae by Steward and Atwood (1963) and suggested the A2 and A3 types facilitated host finding mediating stimuli such as water vapor and temperature while type A1 setae were probably CO_2 sensors.

Upon activation with the prevailing concentration of CO_2 in the upwind search for a host, mosquitoes use other clues to orient to their target, of which convection currents from the body of the host and visual stimuli are the most important. Daykin *et al.*, (1965) pointed out that if the mosquito fails to detect these host emanations and if the degree of CO_2 in the area is maintained, rest behavior is initiated, presumably due to an adaptation to the new concentration of CO_2 . Experimenting on this idea, Simpson and Wright (1967) exploited the likelihood of adapting mosquitoes to low concentrations of some chemical other than CO_2 so that their ability to seek out a host would be impaired. Studies by Khan *et al.*, (1966) seem to suggest that heat and CO_2 are important stimuli used by a mosquito to approach a host in close proximity but that at greater distances odor or a hierarchy of attractant extrudations are utilized. Using a modified New Jersey light trap Snoddy and Hays (1966) successfully trapped Simuliidae with the use of pressurized CO_2 released at a rate of one pound per hour. This rate, which he used in further studies, resulted in optimum

collections of blackflies. Smith (1966) investigated the ability of Simuliidae to locate and discriminate between two sources of CO₂, differing only in their emission concentration levels. His thesis presents a hierarchy of responses for this group of flies predicated on the feeding behavior based on similar pathways previously defined for the Culicidae.

A trap used in population studies of biting flies by Whitself and Schoeppner (1965) consisted of a piece of stove pipe with a sheet of Kraft paper covered with Tanglefoot wrapped around the cylinder. Attractors to produce CO₂ were set inside the piping. Adhesive, sticky surfaces, such as these, have long been used to collect many kinds of insects in general, while other traps, especially those designed for the purpose of monitoring haematophagous population densities, have utilized CO₂ to enhance collections (Nelson, 1965; Collins, 1966; Thompson, 1967; Gillies and Snow, 1967; Wilson, 1968; Schreck *et al.*, 1970). Fallis *et al.*, (1967) used CO₂ in combination with fan traps, "sticky manikins", and "sticky" flesh-coloured paper cylinders and were successful in capturing large numbers of black-flies, but without the CO₂ few were taken. They suggested that CO₂ and vision were especially significant in the orientation and landing of *Simulium venustum*. Working with the Simuliidae as well, Golini (1970) was able to show various degrees of attraction for certain species to CO₂ with and without a silhouette.

Wright (1968) discussed the normal attack program of a mosquito and suggested that the main guideposts required 'and possibly the only' are CO₂, moisture and warmth. Initially, the author and his

colleagues felt that these insects must have to "smell" quite complex products of a host's metabolism. Mayer and James (1970) suggested that CO₂ exerts its prime effect within the mosquito's nervous system. Kashin (1969) presupposed this neurophysiological basis for CO₂ and advanced an hypothesis to this effect. His theory presents, probably, the first complete explanation of the somewhat anomolous reports on the interactions of CO₂ as an irritant to mosquitoes, as Willis and Roth (1952) observed. Carbon dioxide when combined with a heated dummy increased its attractiveness (Brown, 1951a) and CO₂ was thought to be 'intrinsically' more attractive (Willis and Roth, 1952) than heat alone when supplied to the dummy.

Essentially there is confirming evidence that CO₂ is used by mosquitoes to facilitate orientation, distant or medium range, to the host (Gillis and Wilkes, 1969, 1970; Snow, 1970) and that, in general, CO₂ is an activator for these and other biting flies (Gouck and Gilbert, 1962; Mayor and James, 1969; Snow *et al.*, 1968; Acree *et al.*, 1968; Müller, 1968; Daykin, 1967; Khan *et al.*, 1966, 1967; Khan and Maibach, 1966; Smith *et al.*, 1970). In addition to behavioral evidence of CO₂ stimulation, physiological studies have located CO₂ receptors for several species of biting Diptera (Roth, 1951; Willis and Roth, 1952; Rahm, 1958a; Bäessler, 1958; Clements, 1963; Kellogg, 1970; Omer and Gillies, 1971; McIver and Charlton, 1970).

ii.) Smell (chemical host emanations other than CO₂)

In increasing order of importance, moisture, warmth and CO₂ are basic elements involved in the attraction of mosquitoes to a host (Brown, 1966), and amino acids and estrogens comprise a group of

compounds capable of enhancing the basic attractants. It appears that different host selection by mosquitoes has been determined to a great extent by the different emissions of amino acids and estrogens of the hosts' bodies.

Schaerffenberg and Kupka (1951) found mixtures of alanine and cystine to be attractive to *Culex pipiens*, while single compounds of tyrosine and threonine were attractive to *A. aegypti*. Brown and Carmichael (1961) indicated that alanine and arginine were also active. Lipsitz and Brown (1964) tested 26 amino acids and found that the most attractive was L-lysine, but that the degree of mosquito receptivity was directly proportional to the amount of uncombined CO₂ it adsorbed. The amino acids apparently act as a vehicle for CO₂. Additional evidence as to the attractiveness of amino acid compounds was provided by Roessler and Brown (1964), who also showed that estrogens were associated factors, possibly having an influence in the differential attractiveness of men and particularly of women throughout their menstrual cycle. Steward and Atwood (1963), following extirpation of various antennal segments of mosquitoes, concluded that type-1 sensillae were sensitive to human hand emanations. Smith *et al.*, (1970) and Acree *et al.*, (1968) both agreed that L (+)- lactic acid was one of the chemicals that were attractants for *A. aegypti*, while Ikeshoji *et al.* (1963) and Ikeshoji (1967) found that certain amino acids such as methionine and its metabolites were significant enhancers in the attractiveness of *Culex pipiens pallens* to mice.

For the Simuliidae, Lowther and Wood (1964) speculated that *Simulium euryadminiculum* Davies might detect attractant stimuli

emanating from its specific host, the common loon, *Gavia immer* Brünnich, in the water of a lacustrine habitat through tarsal chemoreceptors. Several workers have utilized ether extracts of the uropygial gland of this and other birds to attract ornithophilic simuliids to a collecting station (Bennett *et al.*, 1972; Fallis and Smith, 1965).

Studies on the biting habits of Tabanidae in the British Cameroons, Duke (1955) observed a unique attraction of *Chrysops silacea* to smoke from wood fires prevalent in and around the dwellings of that area. He postulated that the smoke stimulates the tabanid to search out a blood meal and is responsible for increasing the liability of certain individuals (especially natives, whose bodies often emanate the acrid smell of smoke) to be bitten.

II. HYGROSCOPIC

i.) Moisture (relative humidity; water vapor)

Brown *et al.*, (1951) and Peterson and Brown (1951) showed the importance of moisture as one of the factors attracting *A. aegypti* to human bodies. Brown (1951a, 1951b) concluded that when ambient temperatures exceeded 60°F, moisture enhanced the attractiveness of a warm body and was a major attractant factor at these temperatures. Laboratory tests performed on female *Anopheles* mosquitoes by van Thiel and Laarman (1954) also indicated that warmth and humidity were the primary attracting factors. Smart and Brown (1956) noted that human hands with low output of moisture were more attractive to *A. aegypti* than those of high moisture output, a phenomenon which Rahm (1956) tested and found that hands emitted more humidity than arms and were

far more attractive than arms, but that moistening of an arm increased its attractiveness. Rahm (1957a, 1957b) and Parker (1952) also considered mosquito reactions to humidity. Using a dual-port olfactometer, Skinner *et al.*, (1965) showed that air passing over human body sweat was more attractive than that of a control stream of air and concluded in agreement with Brown (1951a) that sweat attracts at low vapor concentration but repels at a high concentration. Roth and Willis (1952) studied the reactions of *A. aegypti* to humidity in an olfactometer and concluded, after removal of certain segments of the insect's antennae, that loci of the hygroreceptors was indeed the site of the antennae. Although their results were difficult to interpret, Frings and Hamrum (1950) reported that a water-vapor sense did exist in the antennae but that it was not the sole loci of these receptors. However, Bar-zeev (1960) and Krafzur (1971) demonstrated various degrees of control upon the thoracic spiracles of *A. aegypti* and *A. triseriatus* and noted that apart from the inherent effect of water balance upon the degree of control, relative humidity itself exerts a direct, sensory effect on the thoracic spiracles. Thus spiracular behavior becomes quickly attenuated in low humidity in comparison with that in high humidities. Roth (1951) had assigned the role of water vapor detection to the antennae, as did Ismail (1962), but Kellogg (1970) thought it possible that another hygro-receptor could be present on another part of the insect's body and he demonstrated that antennae A3 type sensilla of *A. aegypti* responded to water vapor and low relative humidities.

Platt *et al.*, (1957) observed that relative humidity, when compared with vapor pressure and absolute humidity, was a critical

factor. Although visual cues are used by mosquitoes to locate a host, Wood and Wright (1968) concluded that warmth and moisture remained unchallenged as factors mainly responsible for *A. aegypti* responses to an inanimate host. Wright (1968) discussed the normal attack program of a mosquito and suggested that the main guideposts, and probably the only ones, are carbon dioxide, moisture and warmth. Essentially, though, most data compiled on the effect of hygroscopic factors are limited to mosquito attraction involved with near-host orientation (such as warm, moist convection currents rising from a host's surface), the ambient CO₂ level stimulates the insect to fly in search of a host (Khan *et al.*, 1966; Wright and Kellogg, 1962a; Burgess, 1959). Carbon dioxide, it seems, synergizes the attraction to submaximal factors associated with a host and that it exerts an effect on the central nervous system (CNS) of the mosquito which releases an anemotactic flight directly to the host (Mayor and James, 1970), and that moisture emanating from the host also stimulates the fly's central nervous system on near-orientation in the presence of CO₂ (Kashin, 1969). Unfortunately, the many types of olfactometers used in these experiments have caused inconsistencies which still prevail as to the action and interaction of CO₂, warmth and moisture *inter alia* in mosquito attraction (Mayor and James, 1969).

Since 1948, Brown (1966) and his workers have been determining the factors attractive to mosquitoes. He states that "... of the air-borne factors investigated ... moisture proved to be the most powerful single factor" (p. 249), being most attractive in a hot, dry environment. There is agreement amongst most workers that while the relative humidity is below 100%, moist air is attractive to mosquitoes (Brown, 1966).

For the Simuliidae, Edmund (1952) observed that the number of black-flies landing and biting was dependent not only on the number of flies in the "attracted population", but also upon weather conditions. Calculating landing/attracted and biting/landing ratios, he found the former was least and the latter greatest when the temperature and relative humidity favored a large "attracted population". In addition, the effect of temperature was modified by changes in relative humidity. Edmund suggested it would be practical to predict black-fly activity on the basis of meteorological forecasts.

III. THERMAL

i.) Warmth (temperature gradients)

Brown (1966) considered that warmth was only less important to moisture in their search for attractive factors in mosquito orientation to hosts and that a combination of warmth and moisture was greatly attractive. Many other workers concluded similarly (van Thiel and Laarman, 1954; Dethier, 1954; Parker, 1952; Rahm, 1957a, 1957b, 1958b; Laarman, 1958; Burgess, 1959; Kalmus and Hocking, 1960; Kellogg and Wright, 1962a, 1962b; Wright and Kellogg, 1962a, 1962b; Wright, 1962; Wright *et al.*, 1964, (1965); Daykin *et al.*, 1965; Khan *et al.*, 1966; Khan and Mai-bach, 1966; Gilbert *et al.*, 1966). Recently, further experiments on these factors have invariably shown the specific attraction of these factors limited to mosquitoes (Strauss *et al.*, 1968; Wright, 1968; Kashin, 1969), and that warmth plus humidity appear to be the main factors governing the attraction of mosquito spp. such as *A. aegypti* to an inanimate host, inducing landing behavior (Wood and Wright, 1968).

ii.) Convective and radiant heat

Peterson and Brown (1951) established that the attractiveness of dry objects upon heating to temperatures not exceeding 110°F. was due to convective and not radiant heat, and while it appeared that mosquitoes might be able to respond to radiant heat, the responses were due primarily to the convection currents which the warmth and humidity generated (Wood and Wright, 1968). The directional guidance of mosquito behavior appears to be provided by the movement of convection currents from living hosts (Daykin *et al.*, 1965; Laarman, 1958), the responses to which are mediated by vision or a recognition of a warm, wet air current (Kellogg and Wright, 1962b; Wright *et al.*, 1964 (1965)). Thus, in addition to activation by CO₂, landing of mosquitoes on hosts is guided mainly by the local changes in temperature, relative humidity and reinforced by visual patterns (Wright, 1968).

For the Simuliidae, heat appears to have insignificant effects on the attraction of certain groups or ornithophilic species but may have similar effects as those for mosquitoes, though less pronounced, for a mammalophilic simuliid such as *S. venustum* (Fallis *et al.*, 1967).

Using silhouette traps, basically similar to helio-thermal traps of the Thorsteinson design for tabanids (Thorsteinson, 1958), Fredeen (1961) observed that solar heat absorbed by these traps contributed to their ability to attract black-flies. The responses of tabanids to radiant energy was, in fact, later alluded to (Bracken *et al.*, 1962). It is interesting in this respect that Wenk (1962) demonstrated the importance of warm upward currents for host finding for black-flies.

IV. VISUAL

i. Color

The importance of visual stimuli for the Simuliidae was illustrated by Davies' (1951, (1960) 1961) observations that *S. venustum* landed frequently on colored blue and red-purple cloths that had the least amount of total reflected light. He also noted that among dark cloths, those with the highest reflectances in the ultra-violet-blue region of the spectrum were most attractive, a phenomenon which Davies and Williams (1957) utilized to enhance simuliid collections in their ultra-violet traps. Golini (1970) tested the responses of simuliids in both Canada and Norway and found they were attracted more to a silhouette with a low total reflectance of light than to one with a high value. Without the use of a CO₂ stimulant, Fredeen (1961) was able to attract significant numbers of *S. arcticum* to his 'cow' silhouettes which he covered with dark-brown plywood and dark-blue denim cloth. However, the reverse seems to be the case for ovipositing Simuliidae. More eggs are deposited on colored sticks with the highest reflected light (such as that from yellow stakes - Peschken and Thorsteinson, 1965) than on those with low reflection (black and blue ones), a fact with which Golini's (1970) data agreed.

Additional color attraction data for black-flies was promulgated in Peschken's (1960) thesis. He found that simuliids were attracted to black, blue and red spheres more so than to yellow and green. Fedder and Alekseyev (1965) also found black to be the most attractive for the Simuliidae in the USSR. Bennett *et al.* (1972) also indicated the importance of color in the visual attraction and landing responses

of *S. euryaminioulum* to inanimate silhouettes, a matter which Lowther and Wood (1964) previously postulated for the same simuliid species.

Evaluating the landing responses of some Canadian mosquitoes to certain colored fabrics, Brown's (1951a, 1954) findings were essentially the same i.e. attractiveness varied inversely with cloth reflectivity or brightness between 625 and 475 $m\mu$ wavelength, but that the textures of the cloths tended to obscure this generalization. DeLong (1954) considered an increase of mosquito activity to spectral beams and color filters within the 364-400 $m\mu$ range. Smart and Brown (1956) studied the effect of skin hue indicating an increased response of adult *Aedes* mosquitoes to Negroes, Oriental and Caucasians, respectively. On the other hand, Freyvogal (1961) neglected skin temperatures and found no difference in the responses of *A. aegypti* to Africans and Europeans. Sippel and Brown (1953) rendered objects more attractive by an increase in black-white interface in a checkerboard pattern, a principle which Haufe and Burgess (1960) employed in their black and white mosquito traps, and postulated that this visual pattern was superior to competing patterns in the environment. Using a similar pattern, Harwood (1961) attracted many kinds of Diptera. It was observed that when a mouse was exposed in a 10-foot square cage of mosquitoes, the particular visual factors were no less powerful than the airborne ones (Brown, 1966). Other studies have shown the importance of color in the guidance of mosquitoes to a target surface (Kalmus and Hocking, 1960; Kellogg and Wright, 1962a, 1962b; Wright and Kellogg, 1962a; Christophers, 1960; Hecht and Hernandez-Corzo, 1963; O'Gower, 1963; Barr *et al.*, 1963; Fedder and Alekseyev, 1965; Snow, 1971; Platt *et al.*, 1957).

Several investigators on tabanid behaviour have conclusively shown their attraction to black, glossy spheres and other colored objects in the field (Bracken *et al.*, 1962; Hanec and Bracken, 1962; Bracken and Thorsteinson, 1965; Thorsteinson *et al.*, 1966; Bracken, 1960). Modifications in the Manitoba Traps as described by Thorsteinson *et al.* (1965) were made by Granger (1970) for trapping Salt Marsh Greenhead Flies and observed that dark-brown and black were most attractive but that black was not as effective as it had been for other investigators. Other field studies on the Tabanidae revealed that red and black spheres (balloons) were 45 times more attractive than an adult human (Snoddy, 1970). It is interesting, and contrary to mosquito attraction, that Bracken and Thorsteinson (1965) found a drastic reduction of attractiveness to tabanids if a target was designed with equal black and white stripes.

ii.) Movement

Investigation on the sensory behavior of black-flies by Peschken (1960) indicated that movement underneath a modified 'helio-thermal' trap decreased the number of flies captured. Wenk (1963), however, found that both mammalophilic and ornithophilic feeding Simuliidae were attracted to 'projecting parts' of his silhouettes that moved. Again, suspended blue and white striped lard tins, which moved by action of the wind on small vanes, rarely caught anything but black-flies (Collins, 1966).

Bracken (1960) provided evidence that motion increased the attractiveness of dark-bodied traps to tabanids. Movement associated

with either inanimate or living subjects was found to double their attractiveness (Sippell and Brown, 1953) while any flicker effect made an object yet more attractive to *A. aegypti* (Brown, 1966). By superimposing visible movement on a dry, cool target of certain tonal contrast, Wood and Wright (1968) could equal the attractiveness of *A. aegypti* to that of an invisible target dissipating warm and moist convection currents.

iii.) Form (shape, area and contour)

Simulium damnosum was observed to orient in relation to the upper-most substrate layer nearest the host rather than at the ground (Duke and Beesley, 1958). Peschken (1960) and Peschken and Thorsteinson (1965) found that black-flies were more attracted to solid contoured silhouettes, such as circles and triangles, than to those with broken outlines, e.g., X, Y-silhouettes. Silhouette traps of the Fredeen (1961) design were effective in capturing large numbers of black-flies which apparently was a direct function of the surface area and size of opening of these traps, regardless of shape. Interesting in this respect, Anderson and Defoliart (1961) showed a preference selection for hosts depending on size. For instance, large mammals were preferred to smaller by simuliids. Golini (1970) also noted this response. Wenk (1963) also studied the attack behavior of Simuliidae and showed they preferred projecting parts of 'host' objects. Both Fallis *et al.* (1967) and Golini (1970) established that certain simuliids required a silhouette in addition to other stimuli such as CO₂ in order to be 'significantly' more attractive. On this basis, Fallis *et al.* (1967) suggested that simuliids could be arranged into groups as indicated by their

responses to olfactory and 'other' stimuli.

Bracken *et al.*, (1962) indicated that shape of target was important and that a sphere was the most attractive silhouette, especially so with an increasing number of reflecting faces (Thorsteinson *et al.*, 1966), but that the attractancy of cylinder decoys decreased with an enlargement of diameter beyond 30.7 cm. (Bracken and Thorsteinson, 1965).

Experimenting with mosquitoes and their preferred hosts, Downe (1960) suggested that the increase in number of flies feeding was proportional to the host body area exposed. Wright and Kellogg (1962a) explained that, apart from the visual difference, the main distinction between a large and a small target was in the size or extent of the convection currents emanating from it. Further evidence for an effect in deciding the choice between alternative hosts when both were present was presented by Muirhead-Thompson (1951). Kellogg and Wright (1962b) identified specific attractive clues for mosquitoes noting that protruding surfaces were more attractive than flat ones. The responses of *A. aegypti* to varying areas of human skin were studied by Khan *et al.*, (1968). They found that both probing and flying increased significantly when the size of the opening to which the area of skin was exposed was increased from one to nine cm. diameter and flying continued to increase up to 15 cm. diameter. Bidlingmayer (1971) concluded that in the absence of the visual attractants, physical objects such as shrub patterns in a field were used in several ways by mosquitoes as guidelines in their flight paths.

V. MECHANICAL

i.) Surface texture

Brown (1951a) found *A. aegypti* to prefer khaki drill or cotton cloth to greenish-khaki nylon cloth. These findings were substantiated later by Kalmus and Hocking (1960) when they established that certain textures seemed to be favored by mosquitoes for settling and resting. For instance, wool jersey, wood and styrofoam was superior to smooth surfaces such as a cellulose acetate sheet and glass in that order. O'Gower (1963) also noted that rough textured surface stimuli were attractive and one of the important factors mediating the consummatory act of ovipositing in certain mosquitoes.

ii.) Atmospheric pressure

Day (1955) described a novel pair of sense organs in the head of adult *A. aegypti* and suggested, on the basis of its particular structure, that it may function as an organ sensitive to changes in pressure, especially during flight. He reported similar organs in other families of Nematoceran Diptera, but the Simuliidae are not mentioned.

Edmund (1952) related the activity of simuliids to meteorological conditions and observed that a rapid fluctuation or sharp drop in barometric pressures, usually accompanying the approach of a thunderstorm, invariably caused great activity.

VI. SOUND

Apart from the mediating factors involved in breeding swarms among male and female culicids, the only reference made to this sense

was that of Bidlingmayer (1967) who realized the possibility of *Ukanotaenia lowii* Theobald to use the calls of frogs and toads as an aid in finding these hosts.

VII. MISCELLANEOUS

Muirhead-Thompson (1951) and Freyvogel (1961) both found a low attractiveness of human infants to *A. aegypti*. Smith (1956) observed that the skin of a 10-year-old boy was twice as attractive as that of a man, age 38. Rahm (1958b) used an olfactometer and found a differential attraction for humans, noting that males were more attractive to *A. aegypti* than females. However, Roessler and Brown (1964) also used an olfactometer and established the importance of estrogens especially those of women. Other research, had demonstrated that, indeed, individuals have a differential attraction to mosquitoes (Woke, 1962). Another method, called 'probing-time' (PT_{50}), was devised by Khan (1965) and Khan and Maibach (1966) to measure the difference in mosquito attraction to certain individuals. Fifty men were significantly more attractive than 50 women but, again, no explanation relating this differential mosquito response was promulgated (Gilbert *et al.*, 1966).

Following the host preferences of *A. aegypti* for several vertebrate hosts, Khan *et al.*, (1970) concluded that the attractiveness of a host may be best stated only in relation to other hosts. They found man to be the most attractive and that among human individuals they observed a significant difference in attractiveness but showed no similar phenomenon among other vertebrate hosts when compared with their own kind.

MATERIALS AND METHODS

A. DESCRIPTION OF THE GENERAL AREA

Pickavance Creek (Fig. 1.) is situated approximately 16 km. west of St. John's, Newfoundland on the Trans Canada Highway. It is a permanent stream with several pools two to four metres by one to two metres, with depths ranging from 10 to 100 cm., large enough to support a population of the endemic trout *Salvelinus fontinalis* (Mitchell) and possibly containing the introduced trout *Salmo trutta* L.

In the summer of 1970 a site was chosen for the experiments, about 60 m. to the south of the T.C.H. directly adjacent to Pickavance Creek. Later in the same year six other sites were chosen, in and around the same area, in the hope that they would produce greater numbers of simuliids. This hope was not realized and the results obtained from these are presented together with those from the initial site. In 1971 only the original site was used. This site was cleared in July 1970, providing a cleared area about 23 m. x 9 m., which was hidden from the highway, making it possible to leave equipment in position throughout the summer.

The boreal forest region is largely characteristic of Canada. Munroe (1956) proposes the inclusion of Newfoundland in this region rather than in the deciduous forest formation, in spite of the presence of many Nova Scotian elements in the fauna and flora. Boreal conifers are dominant in Newfoundland but the dominants comprising the Acadian forest region are absent, providing an almost imperceptible transition

to the boreal forest region.

The area around Pickavance Creek is composed of a mixture of deciduous and coniferous trees of which balsam fir, *Abies balsamea* (L.); white spruce, *Picea glauca* (Moench); black spruce, *Picea mariana* (Mill.) are dominant. In addition white birch, *Betula papyrifera* (Marsh) and mountain maple, *Acer spicatum* Lam. occur. The major shrubs in the area are: Speckled alder, *Alnus rugosa* (Du Roi), (or mountain alder, *Alnus crispa* (Ait.)); pin cherry, *Prunus pensylvanica* L.; northern honeysuckle, *Lonicera villosa* (Michx.); sweetgale, *Myrica gale* L.; northern wild raisin, *Viburnum cassinoides* L., and *Salix* spp.

B. GENERAL COLLECTING METHODS

Only those techniques used in all experiments will be described here. Any specific technique unique to one experiment is described in the account of that experiment.

Carbon Dioxide: It is well known that carbon dioxide is an efficient attractant for an abundance of haematophagous Diptera (Rudolfs, 1922; Brown 1951a; Reeves, 1951, 1953; Kellogg and Wright, 1962b; Fallis and Smith 1965; Nelson, 1965; Smith, 1966; Snoddy and Hays, 1966; Thompson, 1967; Fallis *et al.*, 1967; Gillies and Snow, 1967; Anderson and Otkowski, 1968; Kashin, 1969; Mayer and James, 1969, 1970; Schreck *et al.*, 1970; Golini, 1970; Roberts, 1970, 1971). Employing steel cylinders containing compressed CO₂, the gaseous flow was controlled first through a regulator¹ which was attached directly to the outlet valve of the

¹L.A. Carbon Dioxide Regulator model #16101597; Canadian Liquid Air Ltd.

cylinder, and later through a flow metre² which made finer control of the gas flow possible. A seven metre length of tubing³ connected the two controls, while a second 18 m. length of tubing led the gas from the flow metre to the site.

The attraction of CO₂ for simuliids, unless otherwise noted, will be understood as a 'releaser' since the Simuliidae use olfactory stimuli from the host in distance orientation.

Stimulus Cards: These were constructed from sheets of 14 ply display cardboard (71 x 112 cm), variously colored. The color cardboards consisted of: a) Dull Coat Expo Blue #1968⁴; b) Dull Coat Expo Red #1967; c) Dull Coat Canary #1911 ('yellow'). The neutral colored cardboards were: d) Dull Coat Black #1909; e) Dull Coat White #1900. Samples of these boards were compared with Munsell⁵ stock color, matte finish, standards at the Munsell Color Company Laboratories, the results of which are given in Table I.

Five simple two-dimensional geometric forms were calculated to approximately equivalent areas and cut from the five possible colors as indicated above. The five forms were: a) Disc - radius 14.35 cm.; b) Square - 25.4 x 25.4 cm. (Fig. 2c); c) Rectangle - 15.24 x 41.91 cm. (Fig. 2a); d) Triangle - base 42.32 cm., height 30.48 cm. (Fig. 2b); e) Star - superimposed 'd' triangles. As these cardboards were colored

²Brooks Type 1355-01A1FZZ; Brooks Instrument Division, Emerson Electric.

³FISHERbrand^R Tygon tubing; a crystal clear, flexible, tubing of modified, plasticized polyvinyl chloride. Fisher Scientific Co. Ltd.

⁴Peterboro Cardboards; Card and Paper Works Limited.

⁵Munsell Color Company, Inc.

on one side only, similar forms had to be glued back-to-back.

Exposure format for each collection period consisted of testing both variables at any given time with either one of the variables, viz., color or form, constant. For instance, on June 3 two tests were conducted using the square form silhouette constant, with a variation in the five colours. When color was common the five geometric forms were varied.

Display Platforms: A display platform consisted of a half-inch plywood centre-piece cut in the shape of a pentagon, each of its five points measuring 25.4 cm. from the centre. Five wooden phalanges, each 60.96 cm. long, 6.35 cm. wide and 1.91 cm. thick, extended from the centre-piece to which they were fastened by door hinges to facilitate folding of the platform for handling and storage. The centre-piece made it possible to position the phalanges equidistantly. To the distal end of each phalange, clipboards, sawn of 5.08 cm. above the metal clip, were fastened perpendicularly by means of wood screws. These clipboards served to hold the stimulus cards upright when exposed in an experiment.

The platform was mounted above the ground, using two lengths of towel rack piping. A 7.6 cm. length was fastened to the underside of the display platform and a 1 m. length was set in the ground. The diameters of the two pipes were such that it was possible to fit the one attached to the platform over the one in the ground. This allowed for a 360° rotation of the platform in either direction (Figs. 2a, b, c). However, the piping rusted during the winter, prohibiting free rotation of the platform and an alternative stand had to be devised for use in

1971. A laboratory stool was adapted so that the platform was approximately 1 m. from the ground, a combination of nuts and bolts allowing free rotation of it (Fig. 3).

Adhesion: Tanglefoot⁶ was applied, from aerosol containers, to the colored surface of each of the stimulus cards to trap the simuliids where they landed. Visual examination indicated that the white cardboard darkened slightly after several coatings, but did not modify any of the other original colors. Simuliids trapped on the stimulus cards were removed later in the laboratory.

Saran Wrap⁷: At the end of exposure all stimulus cards were removed from the display platforms and covered with a single layer of saran wrap for protection of the specimens in transport to the laboratory, where the wrapping could be peeled off without disturbing the position of the simuliids (Fig. 4).

Cleaning technique: Specimens were removed from the cards by placing a drop of xylene on each, removed from the cards with forceps and placed into xylene in watchglasses to dissolve all Tanglefoot, and then stored in 70% ethanol. After this treatment the flies could be identified relatively easily.

Sweep-net collections: After each exposure and covering of the stimulus cards the emission of the CO₂ was continued, to establish the composition

⁶Bird Tanglefoot is a non-drying compound (active ingredients: 48.5% polybutenes; 1.5% hydrogenated castor oil; 50% inert ingredients), which adheres to all surfaces, and retains its soft, sticky elasticity, such that applications will last for months under average conditions. The Tanglefoot Company.

⁷Saran Wrap^R. The Dow Chemical Company.

of the simuliid population that was attracted to the CO_2 by means of sweep-net collections. This involved 40 standard figure-eight sweeps of a net about the author. Live simuliids were collected in an aspirator and placed in cardboard ice-cream containers of a standard C.D.C. design (Collins, 1963). Upon return to the laboratory, the simuliids were anesthetized with chloroform and identified while they were still dry. Following this each specimen was first placed in xylene for several minutes and next in 70% ethanol solution, similar to the technique described (*vide ut supra*). These specimens were again examined to note any inherent changes in body color as it was thought that xylene washings might darken and hence discolor certain taxonomic features.

Meteorology: Temperatures were recorded before and after each exposure from early June to the latter half of August during both 1970 and 1971. During 1970 reflected light readings were taken at the centre of the display platforms before and after each exposure test with a Gossen Lunasix exposure meter. In 1971 wind speeds were monitored with a sensitive cup anemometer set one metre above ground level. Since experiments were carried out when there was little wind, measured wind speeds seldom reached one metre per second and often averaged 30 cm. per second for several hours, (Fig. 5). A glass cylinder containing a wooden rod, to which was attached a drinking straw, was inserted in a two-hole rubber stopper and mounted on a tripod. A white cardboard disc marked in compass points recorded wind direction as the straw indicated, (Fig. 5).

C. SPECIFIC COLLECTING METHODS

I. The influence of color and form on the attraction of mammalophilic Simuliidae.

When a single exposure platform was first employed to display stimulus cards during 1970, CO₂ was released at a rate of 150 cc./min., from the platform centre. The CO₂ cylinder was hidden in the bush. Each exposure was set at 30 min. during which the platform was rotated at 10 min. intervals to offset any positional effects of the target silhouettes. Apart from the few seconds required to rotate the platform, the author spent the exposure period out of range.

It took approximately 60 sec. to secure the stimulus cards in their respective clamps, during which time no appreciable attraction of the flies to the author and hence the targets, was noted. The author was exposed for a similar period when covering the cards with saran wrap, following which another set of stimulus cards were secured to the clamps, subsequently initiating a second period of exposure.

During the latter half of 1970 the employment of a second exposure platform facilitated an increase in exposure time to 50 min., and was similarly rotated at equal intervals of approximately 15 min. duration. Essentially the same procedure was incorporated as with one platform except that the CO₂ outlet was repositioned from the centre of the platform to a point four metres away, up-wind, midway between the two platforms. The platforms stood two metres apart.

In 1971 the display technique was modified to incorporate the use of all stimulus cards at the same time. This involved the systematic

presentation of 25 stimulus cards viz. five colors x five forms, during any one exposure period. The new method provided equivalent conditions and a more valuable means of standardization. Moreover, if vision does play a major role in the host-seeking behavior of the Simuliidae, then such an experiment could test their discriminating capacities for certain preferred color-form stimuli.

To accommodate the 25 color-form stimuli, the test area was cleared according to the measurements outlined for the experimental site (*vide ut supra*). Twenty-five, 5.08 x 5.08 cm. stakes, standing one metre high, were erected at 1.5 metre intervals forming a 7.62 m. x 7.62 m. grid. (Fig. 6). A 7.62 cm. piece was cut from the top of each and an ACCO clamp was attached to each. To secure the clamp section to the stake, a bolt which extended 1.27 cm. from the top to allow the fitting of a wingnut (Fig. 4). The wingnut accommodated rotation of the attached top piece as was desired with changes in wind direction; each stimulus card being held firmly to the stake by means of the clamp. Essentially, this design served a similar function as that of the rotating display platforms. Again as before, a CO₂ source was placed up-wind four metres from the nearest row of stimulus cards on the grid. Emission of CO₂ was controlled by a regulator⁸, attached directly to the outlet valve on the cylinder and two metres of tubing led the gaseous flow to the grid site, at a maximum rate of 1500 cc./min.

Two hours was the maximum exposure time, at a time of day derived from Standard Time data of sunrise and sunset as supplied from

⁸Hudson Type OT-302-B Therapy Oxygen Regulator; Hudson Oxygen Therapy Sales Co.

charts recorded in the Observer Handbook 1971 (Royal Astronomical Society of Canada, 1971). Obtaining the real times from these charts, for sunrise and sunset on any particular day, the mid-point of these two daily occurring phenomena was calculated and referred to as Solar Noon. A real time relating to this point could then be chosen to initiate a test period, to which subsequent testing was, as well, adhered to. This method provided a means of similar effects of sunlight on all targets on each of the days chosen to expose the color-form experiments.

The format of exposure of the 25 color-form stimuli provided each card a number from one to 25, assigned and arranged in order with reference to tables of random numbers. Each two hour test period involved a new order as referred to in these tables. In the field each test followed a systematic placement of cards on the stakes at the grid, with stake position and card number being recorded.

One extra test was carried out following the format as described above, whose function was to test the landing responses of Simuliidae to a horizontal display of 25 stimulus cards. Holes were punched in the centre of each card to facilitate fitting on the inset bolt of each stake at the grid (Fig. 7). Carbon dioxide was released in the usual manner for a period of two hours after which the gas was turned off and the silhouettes left in position over-night. These were recovered 24 hours later.

In addition to the above experiments, two further experiments were conducted to test the color responses of the Simuliidae under field conditions. The first of these involved construction of a

'projection booth' of a suitable size to contain a slide projector and a 'screen port'. This consisted of a rectangular box, made of 2.54 cm. square wooded frames, 13.97 cm. in length and 3.81 x 1.27 cm. in cross-section, and sealed except for both end ports with white diffusible cardboard, glued and stapled to the frames. At one port, .62 cm. plywood screen 76.2 x 60.96 cm. was inserted, containing 10 smaller 3.81 cm. square ports, to which was secured small cardboard holders to support ten Kodak Wrattin Gelatin Filters⁹ of specifically known ranges of spectrum emission (Table II). To protect these delicate filters, diaphanous acetate plates were held to the box frame by mirror clips. This enabled the plates to be removed rapidly and the filters arranged following each test period, according to tables of random numbers. Tanglefoot could be applied directly to the acetate plates. At the opposite end of the booth, a simple 300 watt slide projector¹⁰ with lamp¹¹, was located and connected to a portable generator¹² as its electrical source. A CO₂ source was set at approximately two metres in front of the screen port, (Fig. 8a and b).

The second experiment involved the exposition of 7.62 x 12.7 cm. Munsell Color Standards, Matte Finish Papers, in identifiable colors according to the Munsell system of color notation. This system identifies the standards in terms of three attributes: hue, value and

⁹Eastman Kodak Company.

¹⁰Bell & Howell, TDC 300.

¹¹CMV CMT, 300 watts, 120 v., Sylvania Projector Lamp; Sylvania Electric (Canada) Ltd.

¹²Honda Model E-300 Portable Generator; Honda Motor Co. Ltd.

chroma. The hue notation is based on the ten major hues such as Red, Yellow-Red, Yellow, Green-Yellow, etc., while the value notation indicates a 'degree of lightness or darkness' associated with a color in relation to a neutral grey scale and the chroma notation refers to the strength or saturation of a color sample. Accordingly, the experiment followed two procedures: a) to test a combination of chroma (color) and achroma (neutral) standards with respect to the landing responses of Simuliidae. This comprised an exposure of six achroma standards viz. N 2/, N 4/, N 5/, N 6/, N 7.5/, and N 9.5/,¹³ along with a blue chroma standard viz. 5B 6/6 having a value notation (6/) equivalent to the value notation of one of the neutral greys viz. 5B 6/6 vs. N 6/. Other chroma standards used for additional comparisons were: 5B 2.5/1, 5B 9/1, 7.5B 4/6, and 10B 6/2. b) by varying hue, value and chroma, it was possible to test which of these attributes is most attractive to the simuliids coming to land. For instance, hue could be varied and the value and chroma notations held constant viz. 5B 6/1, 10B 6/1, 2.5B 6/2;¹⁴ or value could be varied with hue and chroma remaining the same, viz. 5B 6/1, 5B 9/1, 5B 2.5/1; and again chroma in variation with hue and value constant, viz. 5B 6/1, 5B 6/6.¹⁵ Luminous reflectance data for the neutral standards are given in Table III.

¹³The complete Munsell notation for a chromatic color is written symbolically: H V/C; while the notation for a neutral, achromatic color is written: N V/.

¹⁴2.5B 6/1 unavailable from the Munsell Color Co. Inc.

¹⁵No further chroma variation was possible due to the fact that 'strong' chroma were unavailable from Munsell.

Both tests were exposed together (Fig. 9). Clothespins glued to wooden strippings held the standards vertical, which in turn were secured to stakes with clamps, at the grid. To protect the surface facings of each standard, which were only thin-film inks, clear polyethylene bags could be slipped over each, and Tanglefoot applied directly. A CO₂ source was located four metres up-wind between the two stakes and following completion of each test the bag coverings containing simuliids were turned inside-out and sealed with an elastic band.

II. The influence of area on the attraction of mammalophilic Simuliidae.

From results obtained following 1970 testing it was apparent that black colored discs trapped more simuliids than any other exposed color-form target. To test the landing responses of simuliids to black disc forms varying proportionately in area, additional discs, based on the original disc stimulus card, were cut, such that the areas varied in the proportions 1:2:4, viz., radius 1 = 10.14 cm.; r2 = 14.35 cm.; r3 = 20.27 cm. The discs were exposed on two platforms of the 1971 design and each test period was set for a duration of 60 minutes. Carbon dioxide emission and all other procedure as previously described were followed (Fig. 10).

III. The influence of colors and carbon dioxide emission rates on the attraction of mammalophilic Simuliidae.

Four formats were followed to test the ability of simuliids to discriminate attractive olfactory stimuli when exposed in combination with an attractive blue and a non-attractive yellow visual target

stimulus, viz. a) two blue discs (Figs. 11b, c) each with a source of CO_2 of equal concentration; b) a blue disc combined with a yellow disc (Figs. 12 a, b, c, d) each with a CO_2 source of equal concentration; c) two blue discs (Figs. 11b, c) each with a CO_2 source of differing concentrations; d) a blue disc and a yellow disc (Figs. 12 a, b, c, d) each with a CO_2 source differing in concentrations.

A clothesline was strung, North-South, approximately three metres above ground level, over the grid site, a distance of 20 m., attached to spruce trees (Fig. 11 a). Where this line intersected the grid centre, 30.5 cm. graduations were marked on the line for a distance of 310 cm. Stimulus cards were attached to the clothesline and to the ground by means of clamps and cords, so that they were exposed one metre above ground level and each could be moved along the line in either direction. Carbon dioxide was released from the main regulator on the CO_2 tank at 1500 cc/min. but a sensitive plastic physiological Y-tube split the flow, one vein leading directly to one target while the other led to a flow meter which could control release of the gas at 10 cc/min. to the other target disc. Both tubing veins were tied over the clothesline so that each outlet extruded CO_2 at the centre of each target; all apparatus could be moved simultaneously (Fig. 11c). When equal concentrations of CO_2 were required at each target, the flow meter was disconnected. Each test period was set at 40 min. maximum, during which time the targets, initially set a distance of 310 cm. apart, were moved closer together at time intervals of 10 min. until they were 30.5 cm. apart from centre to centre, viz. 310 cm, 210 cm, 120 cm, 91.5 cm. 60 cm and 30.5 cm. apart. All other procedures followed normal routine.

RESULTS

A report on the collections of black-flies from insular Newfoundland by Shewell (1957) represents the only previous record of this group of haematophagous Diptera prior to those recently listed for the Avalon Peninsula by Pickavance *et al.*, (1970). Subsequently, the following list of mammalophilic simuliids was recorded and identified from 'silhouette-traps' and from standard sweep net collections at the experimental site at Pickavance Creek, Avalon Peninsula, during the May-September field seasons for the years 1970 and 1971.

- a. The *Prosimulium mixtum* complex
- b. *Cnephia (Stegopterna) mutata* (Malloch)
- c. The *Simulium venustum* complex
- d. *Simulium (Psilozia) vittatum* (Zetterstedt)

Minor numbers of ornithophilic feeding simuliids were collected on the 'silhouette-traps'. In view of the low numbers involved, which were statistically insignificant, it was deemed unnecessary to identify this group of simuliids to species level and accordingly will be referred to in the results as *Eusimulium* spp.

The *Prosimulium mixtum* complex probably includes both *Prosimulium (Prosimulium) mixtum* (Syme and Davies) and *Prosimulium (Prosimulium) fuscum* (Syme and Davies). Although both *Prosimulium* species have been reported (Pickavance *et al.*, 1970) for the Avalon Peninsula, difficulties attendant in the identification of the two species following trapping in Tanglefoot and washings in xylene made

accurate determinations impossible, so that both species are herein referred to as a single group. Studies on the larval populations of simuliids in Pickavance Creek made contemporaneously by Mr. D. J. Lewis (personal communication) indicated that the ratio of *P. mixtum* to *P. fuscum* was 50:50. It is assumed that they were attracted to the target silhouettes in the same proportion.

Adult emergence of the *P. mixtum* complex begins around mid-May, reaching a peak in early June (Fig. 13a, b). The continuing appearance of adult *Prosimulium* in the latter half of July (Fig. 13a, b), may be explained by the slow development of some segments of the larval populations. It is assumed that this group follows a similar life-cycle pattern as in Ontario (Davies *et al.*, 1962) and is univoltine, except that the lower temperatures experienced in the waters of Newfoundland during April-May, may be responsible for the appreciable differences in the timings of some phases of the species life-cycles. For instance, emergence of *P. mixtum* in Ontario in mid-April is at least a month earlier than that for Newfoundland. Lewis suggests that both species are autogenous for the first gonotrophic cycle.

Cnephia mutata has been reported for the Avalon Peninsula by Pickavance *et al.*, (1970), and identified by the present author with confirmation made by Dr. Stephen M. Smith of the University of Waterloo (personal communication). Adults of this species emerge from late May (Fig. 13a) to the middle of June (Fig. 13b) and are univoltine. Similar to the *P. mixtum* complex emergence, slow development may occur in some segments of the larval populations, which may

explain the occurrence of adult female *C. mutata* in late July. Assuming a similar pattern that is present in Ontario streams, this species may comprise both a diploid and triploid form, the former emerging in mid-May, and the latter emerging from early May to the end of June, with one in early August in Ontario (Davies *et al.*, 1962). This possibility lends further explanation to adult occurrence in late July (Fig. 13b). A comparison with the numbers of the other simuliid species obtained (Fig. 13a, b) indicates that *C. mutata* is not abundant at Pickavance Creek.

The *Simulium venustum* complex comprises:

Simulium (Simulium) venustum (Say),

Simulium (Simulium) tuberosum (Lundstrom),

Simulium (Simulium) verecundum (Stone and Jamnback).

All three *Simulium* spp. have been recorded from the Avalon Peninsula (Pickavance *et al.*, 1970). Difficulties attendant on the precise separation of the *P. mixtum* complex also apply to the identification of this group, and consequently shall be referred to in the present account as the *Simulium venustum* complex. Stone and Jamnback (1955) refer to this group accordingly. "The three species *S. tuberosum* (Lundstr.), *S. venustum* Say, and *S. verecundum*, n. sp. form a very closely related group, scarcely distinguishable in the pupa, female or male except for the genitalia" (p. 77). According to Pickavance *et al.*, (1970), *S. venustum* is the most frequently encountered man-biting simuliid on the Avalon Peninsula, however it is likely that some of the collections of *S. venustum* from silhouette-traps and sweep net sampling made in the present study may, in fact, comprise

a number of specimens of the other two *Simulium* spp. Lewis (personal communication) records an approximate larval ratio of *S. venustum* to *S. tuberosum* of 70:30, respectively. Several dissections made on material obtained from sweep-net samples, by the author, indicated *S. tuberosum*. On the other hand, immature stages of *S. verecundum* have not been collected in Pickavance Creek.

The *S. venustum* complex is multivoltine, the first generation adults emerging in late May with the adults of the subsequent generation emerging in early July. Both generations overlap during early July (Fig. 13a, b) making it difficult to distinguish between populations. The late summer populations continue to the latter part of August and represent the last adult simuliids present for the summer.

Simulium vittatum was recorded by Pickavance *et al.*, (1970), and collected from silhouette-traps by the present author; the species identification was confirmed by Dr. S. M. Smith (personal communication). Lewis (personal communication) did not record larval stages of this species from Pickavance Creek but has taken larval samples from the Manuals River, which lies approximately 4.8 Km. west of Pickavance Creek on the Trans Canada Highway. It is assumed that adult female *S. vittatum* (and possibly other simuliid species) collected on silhouettes migrated this distance or are otherwise present in other rivulet streams prominent in the areas adjacent to Pickavance Creek. From the collections made from silhouettes, it appears that this species follows a similar timing in the life-cycle phases as recorded for the same species in Ontario (Davies *et al.*, 1962), with the exception that all generations of *S. vittatum* in Ontario are autogenous. The spring generation of females of *S. vittatum* at Pickavance

Creek have been observed to frequently land on the author, but biting and feeding were not noted. On the 17 May, 1971, several female *S. vittatum* were collected as they landed on the author and ovarian dissections indicated all were autogenous, most with mature eggs. Although Pickavance *et al.*, (1970) records six *S. vittatum* females as being caught biting man at Pickavance Creek, it is likely that these collections were made during the latter half of the summer, when the summer generation of 'biting' females of *S. vittatum* are in abundance (Fig. 13a, b).

Experimental Studies

In 1970, from June to mid-July the mean monthly temperatures at the site ranged from 16-23 degrees centigrade, and 25-31 degrees C. during the latter part of July and most of August. Precipitation was extremely low (Meteorological Office, St. John's Airport). The high temperatures and dry conditions reduced Pickavance Creek to a mere trickle and the numbers of simuliids were reduced markedly (Fig. 13a).

In 1971, although temperatures still showed a relative high range, 18-25 degrees C. with some hot periods of 30 degrees C. being recorded, the conditions were generally not as drastic as those experienced in the former season, and except for the *P. miazum* complex population simuliid numbers were considerably higher (Fig. 13b).

EXPERIMENTS AND OBSERVATIONS IN 1970

I. The influence of color and shape on the attraction of mammalophilic Simuliidae.

Simuliid collections were made throughout June and August of 1970 in seven different sites around Pickavance Creek as described previously. The habitat preference of simuliids was not the concern of this study and accordingly data obtained from the 1970 collections were combined for statistical analysis, as it was evident from standardized collections made in 1971 that results showed much the same pattern. It was important that optimal conditions prevailed when simuliid activity was greatest and it became necessary to adhere to a regular routine in the field when the available simuliid population was high. The following were some of the factors influencing simuliid activity and density:

1. Time of day, e.g., few or no simuliids were present between 2100 and 0500 hours, and during the heat of the sun throughout 1000-1500 hours, whereas collections between 1600-2000 hrs. were most profitable.
2. Temperature, e.g., simuliid activity was high between 16 and 25°C, lowering markedly below 15°C.
3. Barometric pressure, e.g., a sudden 'drop' in pressures, which was usually the case prior to a rainfall, invariably enhanced simuliid activity.
4. Wind speeds, e.g., simuliid numbers were high when wind speed remained around 1-2 m/sec.

The collecting methods employed maximum release of CO₂

while the exposure of the maximum number of silhouettes during any one exposure period and the use of the most attractive color-shape silhouette definitely influenced the simuliid catch. It was necessary to employ longer periods for each test period when drought conditions were prevalent in 1970, but despite efforts, simuliid collections were comparatively low.

During June and July, 25 systematic half-hour collections were made using one CO₂-baited exposure platform containing one set or five stimulus cards as described (*vide ut supra*). With the exception of an additional CO₂-baited platform, the only major alteration in the above design was an increase in the exposure period from 30 min. to 50 min. during July 8-20. From July 21 to August 15, exposure periods lasted as long as the author deemed necessary. This involved periods ranging from one hour to seven and a half hours as was the situation on August 29. With these few alterations, aimed at enhancing simuliid collections, statistical analyses of these data were carefully considered so that reasonably standardized results would be obtained. This comprised a statistical three-way analysis of variance (Appendix I) conducted on the basis of a standard number of exposure periods.

It is most evident from data (Fig. 14) that there is a marked differential response to colored silhouettes among simuliids at Pickavance Creek. The *P. mixtum* complex, *C. mutata* and *S. vittatum*, the early spring species, show a preferred landing response to black colored targets, while the *S. venustum* complex consistently landed in large numbers on 'blue' (Munsell purple-blue, Table I) silhouettes. The most attractive colors for all species of Simuliidae were black,

TABLE I.

'Peterborough' Stimulus Cards	'Derived' Munsell Notation	Luminous Reflectance/ Munsell Value (in % relative to MgO)	'Peak' Wavelength (Millimicrons)
No. 1900 Dull Coat White	N 9.15/	81.95	
No. 1911 Dull Coat Canary (Yellow)	7.5 Y 8.6/10.5	70.37	560
No. 1967 Dull Coat Expo Red	7 R 4.6/15	16.37	(580) - 700
No. 1968 Dull Coat Expo Blue	6 PB 3.7/14	10.13	450
No. 1909 Dull Coat Black	N 1.75/	2.53	

** Luminous reflectance for light source "C", equivalent to average north daylight having a color temperature of approximately 6,750 degrees Kelvin.

The Munsell System is based on spacing the colors for their appearance under C.I.E. Source, equivalent to a color temperature of 6,750 degrees Kelvin, which is equal to average North Daylight from a partially overcast sky.

'blue' and red, although 'blue' did not attract large numbers of *Prosimiliids*, while yellow was the least attractive, with few landing on white silhouettes.

A comparison of these data with that of Fig. 16 indicates that the percentage of luminous reflectance of each stimulus card is ^{inversely} proportional to the numbers of simuliids coming to land, viz., a decrease in the luminous reflectance or the silhouette reflecting the least amount of light increases the simuliid catch or attraction. The luminous reflectance data of each stimulus card is provided in Table I. This pattern is quite consistent for each color except that more simuliids landed on white than yellow even though white has a higher value of luminous reflectance. This may be explained by the fact that several applications of Tanglefoot darkened the white cardboards appreciably such that it tended to appear grey in the sunlight. The luminous reflectance value was recorded only for the original white colored surface.

There was no significant difference in the responses of simuliids to the variously shaped silhouettes for both summers. There was some indication from the total numbers of simuliids landing in 1970 (Fig. 15) that discs were preferred and this resulted in their use for experiments designed in 1971. It appears that simuliids do not discriminate between these particular exposed contours. Direct observation however indicated that there was a preponderance of simuliids landing specifically on or near the various 'points-of-convergence', viz., corners of square silhouettes (Fig. 4), and points of the star silhouette, etc.

EXPERIMENTS AND OBSERVATIONS IN 1971

I. The influence of color and shape on the attraction of mammalophilic Simuliidae.

Implementation of a new standardized design in 1971, as described earlier, provided an element of quality which suggested that most of the quantitative data arrived at in 1970 were significant and a comparison of figures for both seasons makes this quite evident. However, the only apparent irregularity in this design was a proportional increase in the number of flies on those rows of stimulus cards, on the grid, nearest the CO₂ source. This was to be expected and it is here pointed out that the Simuliidae showed no degree of preference to the furthest down-wind stakes, i.e., the design did not indicate any particular up-wind positional effects. The orientation of simuliids on the grid depended largely on the color of the silhouettes and their individual position on the grid, apart from the near-orientation effects of the CO₂ as noted above. Reference to Fig. 17 will show this phenomenon clearly. As a function of distance from the CO₂ source, the higher percentage of simuliids were trapped on silhouettes of the nearest row, but as the distance from the gas source was increased, it is noted that color seems to be the deciding factor for that preferred landing position for simuliids at the grid. For example, substantial catches were recorded on 'blue' and red silhouettes, as far as seven metres and seven and three-quarter metres from the CO₂ source, but relatively few simuliids landed on yellow silhouettes only four metres away (Fig. 17). It is possible that the Simuliidae are using their visual capacities to locate an 'attractive' target between four and seven

metres from the CO₂ source during their in-flight or near-orientation behavior. It is evident from Fig. 17 that numbers of simuliids generally diminish in numbers proportionally as the distance from the CO₂ source increases. Results represented in Fig. 17 comprise responses of all four species of Simuliidae at Pickavance Creek, such that it may be reasonable to assume that each species has a particular critical distance from the CO₂ source in which vision is used in near-orientation discrimination of targets.

All 25 silhouettes (viz., five colors x five shapes) were exposed three times on June 4, 15 and July 17. These collection dates were chosen to include (i) the early spring emerging adult Simuliidae, *S. vittatum* (first generation), the *P. mixtum* complex and *C. mutata* and (ii) the subsequent two generations of the *S. venustum* complex and second generation *S. vittatum*.

The landing responses of the simuliids to both color and form targets are presented in Figure 18 and it is clear that each species has a differential response to certain colors, confirming the 1970 results, varying in that *S. vittatum* appeared to have been attracted to red (Fig. 18) rather than black (Fig. 14). Again, as in 1970, simuliids showed no marked or significant difference in the numbers landing on the variously shaped silhouettes (Fig. 19). It is probable that the number of simuliids landing on any particular silhouette is governed by its color, not its shape. The blackflies tend to land at the prominent points of convergence of these silhouettes, a fact noted the previous summer.

Responses of the simuliids to the various percentages of

luminous reflectance (Fig. 16) indicated that the pattern of 1970 was repeated, with one marked difference: Black which has an almost negligible reflectance value (Table I) had fewer flies landing proportionally than that for either red or 'blue'.

Immediately following completion of the July 17 test period, a second experiment utilizing the 25 stimulus cards was initiated. Its function was to test the landing responses of simuliids to a horizontal display of silhouettes (Fig. 7). Carbon dioxide was supplied to the site for a period of two hours, and recovery of the stimuli 24 hours later indicated negligible numbers of Simuliidae. No further tests were conducted, as it was apparent that the display was not attractive to female Simuliidae.

Results recorded (Table II) show the numbers of Simuliidae coming to land at the illuminated screen-port of the projection booth. The majority of simuliids collected following the completion of two test periods, at both red Wrattin filters were too few to warrant any speculation on the significance of these data. However, it was realized that this attempt at projecting light through known color band filters for the purpose of attracting simuliids, represents one of the first of its kind. Problems attendant on technicalities associated with the electrical source and the completion of the fly season subsequently resulted in the discontinuance of the experiment. It was suggested that a practical means was attained with a relative amount of success to bring a laboratory situation into the field.

Unfortunately there was not sufficient time to test all variations of the Munsell system as previously described, as simuliid

TABLE II

Kodak 'Wratten' Filter	Dominant Wave-length (C)	Percent Luminous Reflectance (C)	Total Number Simuliidae Landing (Two test periods)
No. 9 (deep yellow)	574.4	76.6	0
No. 11 (yellowish- green)	550.3	40.2	2
No. 22 (yellow-orange)	595.1	35.8	0
No. 25 (red tricolor)	615.3	14.0	10
No. 29 (deep red tricolor)	631.6	6.3	10
No. 38A (blue)	478.8	17.3	1
No. 45 (blue-green)	481.5	5.2	1
No. 47 (blue tricolor)	463.8	2.8	0
No. 58 (tricolor green)	540.3	23.7	2
No. 70 (dark red)	676.0	0.31	2

populations dwindled, to mark the end of the field season. Accordingly, Table III includes four replicates involving variation of the hues (Table IIIa) and five replicates of the neutral achroma standards in combination with a hue (Table IIIb). The numbers of collected simuliids for the first experiment (Table IIIa) revealed almost equivalent numbers on each of the blue hues. Results obtained from the exposure of the chroma/achroma standards (Table IIIb) show no consistent pattern with those of any previous experiments. For example, there is no apparent association in the numbers of Simuliidae landing and the per cent of reflected light from each stimulus card. However, the colored standard (5B 6/6, Table IIIb) did indicate a high count of simuliids especially when compared to the neutral standard having a similar 'value' attribute, viz., N 6/ .

II. The influence of silhouette size and surface area on the attraction to mammalophilic Simuliidae

An experiment was designed to determine the influence of target area on their attraction to blackflies. Studies in 1970 showed that a preponderance of simuliids were attracted to black silhouettes (Fig. 14), in particular, the disc-shaped ones (Fig. 15). In this study three black, disc-shaped targets of areas in the ratio of 1:2:4 were tested six times. The results (Fig. 20) are presented as the pooled averages of the six replicates. The number of simuliids caught on the three targets was in the ratio 1:1.8:2.7. These results indicate that target sizes are of significance in attracting simuliids at Pickavance Creek and that the total numbers of attracted simuliids increased in proportion to an increase in target area. On the basis of these data,

TABLE III.

Munsell Scale H V/C	Percent Luminous Reflectance	Replicate					Simuliidae Total
		1	2	3	4	5	
a. Hue varied/value and chroma constant							
10 B 6/1		21	0	30	6		57
5 B 6/1		24	6	47	6		83
2.5 B 6/2		13	5	22	10		50
b. Chroma vs. achroma							
N 9.5/ (Ac)	90.01	6	15	6	14	2	43
N 7.5/ (Ac)	50.68	5	12	3	2	0	22
N 6/ (Ac)	30.05	3	4	1	2	2	12
N 5/ (Ac)	19.17	1	3	2	13	4	23
N 4/ (Ac)	12.00	2	19	5	3	1	30
N 2/ (Ac)	3.1	4	3	0	9	7	22
5 B 6/6 (C)	10.13	6	14	1	3	12	36

therefore, it would appear that by increasing the size of a given target surface area one would expect a similar proportionate increase in the numbers of Simuliidae landing.

III. The influence of color and carbon dioxide emission rates on the attraction of mammalophilic Simuliidae

Systematic collections of Simuliidae were carried out on July 17, 18, 21, 22, 1971 (Table IV, V, VI, VII) to test the effect of 'attractive' and 'non-attractive' olfactory and visual stimuli. Realization that certain colors attract simuliids significantly more so than other colors, as indicated by results obtained during 1970 and 1971, prompted several experiments that would test the importance of vision in the near-orientation behavior of Simuliidae.

Smith (1966) demonstrated that simuliids had a profound olfactory ability to discriminate between two sources of CO₂ differing in their release rates, and showed clearly that when two traps emitted equivalent concentrations, equal numbers of simuliids would be attracted. Accordingly, the initial task in this series of investigations was to show that color equivalent target silhouettes, with equal rates of CO₂ emitted beside them, would attract equivalent numbers of Simuliidae. The comparison of the numbers of flies captured on each silhouette has been defined by Smith (1966) as the Net Discrimination Ratio (NDR), viz., the total number of simuliids on 'Blue' targets 1 : the total number of simuliids on 'Blue' targets 2, Table IV. From previous work it has been shown that disc-shaped targets were favorable and that 'blue' was attractive to *S. venustum*, the dominant fly at the time of year the experiments were conducted.

TABLE IV

Olfactory and Visual Discrimination by Mammalophilic Simuliidae as a Function of Distance Between Stimuli (mainly *S. venustum*).

Blue	Blue	Blue	Blue	Blue	Blue	Silhouette Color	
1500	1500	1500	1500	1500	1500	CO ₂ cc./Min.	
124	139	210	118	126	122	No. Flies on T1	
Net Discrimination Ratio 839:823 = 102:1							
	30.5	61.0	91.5	120	210	310	Distance between silhouettes in cm.
	91	181	180	133	91	147	No. Flies on T2
	150	1500	1500	1500	1500	1500	CO ₂ cc./Min.
Blue	Blue	Blue	Blue	Blue	Blue	Silhouette Color	
1.36	0.77	1.17	0.89	1.38	0.83	Discrimination Coefficient # @ T1 / # @ T2	

TABLE V

Olfactory and Visual Discrimination by Mammalophilic Simuliidae as
a Function of Distance Between Stimuli (mainly *S. venustum*)

Yellow	Blue	Yellow	Blue	Yellow	Blue	Silhouette Color
1500	1500	1500	1500	1500	1500	CO ₂ cc./Min.
14	171	18	299	7	56	No. Flies on T1
Net Discrimination Ratio $1,539:127 = 12.12:1$						T1 North
						Distance between silhouettes in cm.
30.5	61.0	91.5	120	210	310	T2 South
213	35	592	33	208	20	No. Flies on T2
1500	1500	1500	1500	1500	1500	CO ₂ cc./Min.
Blue	Yellow	Blue	Yellow	Blue	Yellow	Silhouette Color
15.21	4.89	32.89	9.06	29.71	2.80	Discrimination Coefficient i.e. # @ Blue/# @ Yellow

TABLE VI

Olfactory and Visual Discrimination by Mammalophilic Simuliidae as a Function of Distance Between Stimuli (mainly *S. venustum*).

Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Silhouette Color
1500	1500	10	1500	10	1500	10	1500	CO ₂ cc./Min.
203	135	33	17	29	56	58	98	No. Flies on T1
								Distance between silhouettes in cm.
								61.0
Net Discrimination Ratio $757:698 = 1.08:1$								No. Flies on T2
								234
10	10	1500	10	1500	10	1500	10	CO ₂ cc./Min.
Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Silhouette Color
0.87	1.01	1.64	0.39	1.28	0.56	2.71	1.48	Discrimination Coefficient i.e. #@1500cc/min/#@10cc/min

TABLE VII

Olfactory and Visual Discrimination by Mammalophilic Simuliidae as a Function of Distance Between Stimuli (mainly *S. venustum*).

Yellow	Blue	Yellow	Blue	Yellow	Blue	Blue	Yellow	Silhouette Color
10	10	1500	1500	10	10	1500	1500	CO ₂ cc./Min.
6	168	2	147	0*	25	40	8	No. Flies on T1
Net Discrimination Ratio 643:39 = 16.49:1								T1 North
	30.5	30.5	61.0	91.5	120	150	210	
								T2 South
	65	11	95	4	86	6	0*	
1500	1500	10	10	1500	1500	10	10	CO ₂ cc./Min.
Blue	Yellow	Blue	Yellow	Blue	Yellow	Yellow	Blue	Silhouette Color
10.83	15.27	47.50	36.75	86.0	4.17	40.0	2.13	Discrimination coefficient i.e. # @ Blue / # @ Yellow
*Arbitrarily = 1								

In the first series of experiments, the attractance of blue discs of equal area and with equal flows of CO_2 were compared. The results (Table IV) indicate a NDR of 1.02:1. Clearly, the attractance of the two test discs was identical, and the results were analogous to those obtained by Smith (1966) using clear plastic fan traps.

A second experiment was now designed to test the importance of color. A 'blue' disc of the previous experiment was substituted with a yellow one of equal area, as yellow (Figs. 14, 18) has been shown to be a color of low attractance. The results (Table V) clearly show that simuliids can discriminate between two traps on the basis of their color composition. In each replicate, the yellow silhouette collected fewer flies than the blue, even though both had equal amounts of CO_2 released at their centres at all times. When the targets were in contact (30.5 cm. from centre-to-centre), the flies could discriminate between them. At 310 cm. apart, the discrimination coefficient, i.e., the numbers of simuliids on the 'blue' target/the number of simuliids on the yellow target, is lower. Possibly at distances exceeding this, the simuliids visual discrimination is replaced by reliance on olfactory senses.

It was now decided to investigate the attractance effect of equivalent targets with different quantities of the gas being emitted. Two 'blue' discs were employed with 10 cc/min. of CO_2 released at one trap and 1500 cc/min. at the other, a ratio of CO_2 emission = 1:150. To avoid undesirable position effects, the positions of the CO_2 sources were alternated for each test. The data (Table VI) indicates an equivalence of attraction similar to that observed previously

(Table IV), the numbers of Simuliidae for each trap were approximately equivalent, with an NDR of 1.08:1. Hence it appeared that the simuliids were responding to color independent of olfactory stimuli.

A further experiment was performed, comparing the attractance of a 'blue' disc with that of a yellow disc with each colored target emitting CO_2 at the rate of 10 cc/min. (4X) and 1500 cc/min. (4X). Here again, the CO_2 quantities at each target differed in a 1:150 ratio, and procedure involved an alternation of the relative positions of the targets along with the two CO_2 sources. From the results (Table VII), a clearly marked discrimination of Simuliidae for preferred colors independent of chemical stimuli is demonstrated. As indicated, the NDR is 16.49:1, i.e., the total number of simuliids on 'blue' targets/the total number of simuliids on yellow targets, which if compared with that shown in Table V (NDR of 12.12:1), is greater, even though the yellow target in that test had consistently higher levels of CO_2 concentration exposed with it. Again, in this final test, it appears that in the range 210 cm.-310 cm. distance, separating the targets, the discrimination of the flies tends to break down considerably, possibly critical distances between two targets for visual discrimination of the two subject stimuli.

DISCUSSION

Ethology-oriented studies on Simuliidae have shown a rather elegant format or hierarchy of responses attendant to host location in the field (Smith, 1966; Golini, 1970). The essential factor underlying simuliid's behavior is physiologically based and despite the fact that some species are autogenous (Davies and Peterson, 1956), most require a blood source to complete the primary and later gonotrophic cycles (Davies *et al.*, 1962). The behavior of adult females of most species of Simuliidae is a reflection of this requisite to ovarian development, and their selection of host is mainly restricted to homoiotherms, although a few accounts do report feeding on poikilothermic animals (Davies and Peterson, 1956; Smith 1969).

The host-finding and attacking program associated with the Simuliidae involves a continuum of unit responses dependent on the physiological state and other elements intrinsic to the fly, besides various extrinsic factors related to it's environment. Additional factors combining the exposition and intrinsic makeup of available hosts (e.g., color, shape, size, host extrudations) effectively directs the fly's orientation to host in space of which color, shape, size and host extrudations are a part. Theoretically, a whole chain of stimulus-response reaction occur which eventually leads the insect to the host. Considering this, we may assume that if an experimental study involves only part of these behavioral units and not the complete pathway, it is likely that results would differ on each account. Thus it is paramount that one allows the insect a completion of the full chain of events conducive to a situation that

permits observation in the animal's natural habitat and, unequivocally so, when experimenting the flight paths of Simuliidae.

The appetite and associated 'readiness to act' condition inherent in biting flies is the prime concern of Stage I as presented by Smith (1966). An adult female simuliid, in an appropriate physiological state for a blood meal, may either await potential host stimulation or search for the adequate stimulus situation which allows consummation of the feeding sequence. It may be that both possibilities are operative at one time or another. Smith added that in the Simuliidae selection of particular habitats in which a host would be sought, is an essential element characteristic of this initial phase. He was careful though, not to imply that the presence of a host strictly determined the habitat but that by virtue of a rejection of one host for a preferred host an interruption of the hierarchy of feeding behavior would be involved, indicating that selection is a multi-levelled hierarchy closely related to the insect's host-finding mechanisms. Examining preferred habitats for certain ornithophilic simuliids, Bennett (1960) exposed hosts to these flies in both common and alternate habitats. He demonstrated an almost strict adherence to the characteristic habitat of the simuliids such that placing a suitable host in an alternate situation was ineffective in luring species in significant numbers outside their preferred location. Another example was observed in the behavior of *Simulium (Eusimulium) euryadminiculum* Davies, a highly specific ornithophilic species feeding only on the common loon, *Gavia immer* (Brünnich), in only certain riverain and lacustrine habitats (Davies and Peterson, 1957; Lowther and Wood, 1964).

Thus, Stage I occurs only within the preferred habitat of the species of Simuliidae involved (Smith, 1966).

In 1918, Craig defined animal appetitive behavior and explained that when a consummatory reaction is released "the appetitive behavior ceases and is succeeded by a state of relative rest" (p. 91). Accordingly, Golini (1970), effectively clarified Smith's (1966) terminology in defining Stage I, "appetitive behavior leads not only to habitat selection ... but also to the additional four stages" (pp. 165-166), which included Stage II or upwind orientation, Stage III or near-orientation, Stage IV or landing-behavior and finally Stage V, crawling and burrowing.

In this context, Stage II of the hierarchy involves a stimulus situation which ultimately functions as a releaser. The nature of the stimulus attending elicitation of corresponding host-seeking behavior appears to be related to appropriate olfactory stimuli associated with certain host emanations, of which instances of specific host attractants are rare. For example, *S. euryadminiculum* is capable of locating the source of an odor originating from the uropygial gland of the common loon with considerable accuracy (Smith, 1966). Although Lowther and Wood (1964) believed that this simuliid species could detect and locate its host up-wind as a result of this specific airborne olfactory attractant, Smith has observed it to approach ether extracts of the host's uropygial gland from a down-wind orientation.

Carbon dioxide has been widely employed as a releaser of up-wind oriented behavior for most biting Diptera. Other odors may serve a similar function but specific chemical compounds emanating

from mammalian hosts have not been established, a fact that the synergistic effect of carbon dioxide would seem to obscure. Carbon dioxide is capable of releasing up-wind orientation in all Simuliidae. Specific odors may also function as a releaser as that which is known for *S. euryadminiculum* (Smith, 1966), while carbon dioxide by itself is a poor attractant for this species (Bennett *et al.*, 1972). However it has been pointed out that such a relationship is unique. Golini (1970) investigated the roles of a wind-borne stimulus in the orientation of simuliids to carbon dioxide under field conditions. Using a horizontal rotating wheel, the circumference of which contained vertical pegs, he observed that "most black-flies were caught on the down-wind pegs with a progressive decrease in the number of flies on pegs towards the up-wind side of the wheel" (p. 76). It was proposed for Stage II that the insect makes use of visual clues in up-wind orientation and that the chance of the fly orienting up-wind directly to an odor cloud or carbon dioxide was slight (Smith, 1966). It is possible that carbon dioxide facilitates activation in the initial stages of attraction to a host and it is also conceivable that the maintenance of up-wind flight paths is dependent on constant stimulation by carbon dioxide or other host odors. Kashin (1969) hypothesized the underlying mechanism by which a mosquito locates a host. In his model he proposed that an amino acid, gamma-aminobutyric acid (GABA), which he found in significant quantity in mosquitoes associated with the insect's nervous system, is a synaptic inhibitor which also occurs in the nervous system of many mammals, micro-organisms and plant tissues (Elliott, 1965). Kashin explains that it can combine with CO₂, facilitated by atmospheric moisture, resulting in a carabamino-GABA

compound. Uncombined GABA present in the insect's specialized host-finding apparatus is a neuroinhibitor but when activated by CO₂, atmospheric moisture and moisture emanating from a warm-blooded host, the inhibitory power of GABA is diminished or abolished as a result of the reaction. Increased temperature, however, serves only to increase the breakdown of the carbamino compound into its components. Kashin suggests that activation with CO₂ not only causes excitation but may act as an irritant or noxious stimulus to the mosquito. Thus, upon further excitation flight activity is increased so that upon entering the 'host-stream' the warm updraft of air serves to uncouple GABA from CO₂ and the mosquito becomes less activated and irritated. Now the mosquito is stimulated to seek warmth and once in the host stream all the essentials such as CO₂, warmth and water vapour are available to promote a continual interaction in the neural system between the activation reaction and inhibition ($\text{GABA} + \text{CO}_2 \xrightarrow[\text{(catalyst)}]{\text{H}_2\text{O}} \text{GABA} \cdot \text{CO}_2$). As Kashin points out, the host stream serves to keep the mosquito constantly "primed" and "on target." The stimulating micro-climate and environment at the host's skin surface greatly stimulates further activity to result in probing, tapping of the blood source and further engorgement to repletion which ultimately switches off the initial chain of events. Because such reactions require host moisture, warmth and CO₂, it is unlikely that such a mechanism could be operative very far from the host. Golini (1970) showed that collections of simuliids at distances 10 and 15 feet away from the CO₂ source were few, while Gillies and Wilkes (1970) indicated that certain species of Anopheline mosquitoes detected and responded to CO₂ from less than 15 yards and that the *Culex decens* group the distance was only five yards. Thus, it appears that

long-range orientation is mediated through olfactory cues but visual cues are also employed in directing up-wind orientation.

Near-orientation to host, Smith's (1966) third hierarchal stage, differs from distant orientation to host in that simuliids are apparently capable of responding 'directly' to odor gradients. His thesis clearly demonstrates the ability of the Simuliidae to locate, orient and discriminate between two sources of carbon dioxide on the basis of their gaseous emission rates. An *a priori* experiment by Smith indicated that "identical attractants capture equal numbers of flies" (p. 87). Using miniature suction traps he initiated another test which utilized two CO₂ sources differing in release rates but this time as a function of distance between the two CO₂ sources. A discrimination coefficient was calculated (viz. the higher emission source/the source releasing the lesser amount) which effectively showed that "the discriminating coefficient decreases with decreasing distance between the two traps" and that "the population was still selecting the higher trap when the traps were only one foot apart (centre to centre) and the selection becomes more definite with increasing distance between traps" (p. 90). From these results Smith postulated that simuliids probably did not rely on vision until they were close to the host but his traps or 'silhouettes' did not portray a 'striking' character. Accordingly, he suggested "there exists the possibility that variously colored traps might effect a dissolution of the discrimination when the traps are separated by distances greater than two feet" (p. 125). Such properties of stimuli which Smith refers to as being associated with the landing behavior of

Simuliidae, comprise Stage IV.

Mediated by visual stimuli, the termination of the 'in-flight' orientation of Simuliidae may be classified as 'landing behavior'. However, it is recognized that both vision and olfaction are employed by haematophagous Diptera at this stage but quantitative data is lacking in the degree to which each sensory role plays in addition to the distances at which certain biting flies make use of their sensory equipment. For instance, the Tabanidae are noted to use visual clues at greater distances from a host to guide their attack behavior than those known for the Simuliidae (Smith, 1966).

When a simuliid has located and landed on a host, the final two stages in Smith's hierarchy (Stages V and VI) are taken to completion ensuring feeding. This consummatory act, then, is at the end of a complex series of behavior patterns involved in appetitive behavior or a search for the appetized stimulus--a blood-meal which has obvious survival value for certain biting flies. Engorgement is attained and appetitive behavior is then terminated, a state of relative rest ensuing.

The purpose of this thesis, as has been described (*vide ut supra*), assumes Stage I and II operative for the Simuliidae and reveals the importance of some of the factors influencing the successive two stages. Stage III has been tested to establish the ability of simuliids to locate and orient to two carbon dioxide sources differing in rates of emission when exposed in association with an 'attractive' and a 'non-attractive' colored disc-shaped silhouette. An estimation of the extent to which simuliids use their sensory capacities of olfaction and vision

is an attempt at a dissolution of the elements and the importance of each in mediating the insect's orientation to an inanimate host. Subsequently, Stage IV was experimented to establish (a) the differential landing rates of mammalophilic Simuliidae, and indeed each simuliid species populating the Pickavance Creek area, on variously colored stimuli, viz. 'Peterboro' cardboards, projected Kodak Wrattin gelatin filters and 'Munsell' standards; (b) the effect of similar stimuli differing in shape and contour and (c) the preference for target silhouettes differing in the size of the area exposed in proportionate ratios of 1:2:4 and the importance of these elements presumed associated with a host in nature was attempted through a quantitative analysis of the numbers of black-flies landing on each.

It is herein assumed from the established terminology that one heirarchical stage of the flies' behavior is contingent on the next, in as much as it is the concern of the present text.

The role of various radiation compositions in the visual near-orientation and landing behavior of mammalophilic Simuliidae.

Since the experiments of von Frisch (1958) on the color responses of bees, the idea that insects may in fact discriminate various colors independent of intensity has intrigued many investigators. Publications dealing with the differential landing responses of Simuliidae to color are few. As yet, no proof is available of visual color discrimination in any blood-sucking insect (Hocking, 1971). Studying the attractiveness of colored cloths to simuliids in the field, Davies (1951) observed that the attractancy of a cloth was influenced more by the intensity than by the wavelength of the reflected light

such that when the total intensity of the light reflected from the cloths was high, few flies landed, but when the intensity was low, it stimulated more flies to land. He advised when *S. venustum* was the abundant pest that white clothing would be desirable whereas blue was the least desirable. Following these initial investigations, Davies (1961) used the Munsell color system to standardize his colored stimuli, to test for the attractiveness of each color component separately, viz. hue (wavelength), intensity (lightness) and chroma (purity), to *S. venustum*, a common summer pest in Ontario. He observed that a red-purple hue was most frequented, purple and purple-blue next, with red and blue equally selected by this species. Whereas he acted as host, remaining in the shade (presumably blocked the incident light of the sun) and counting the simuliids simultaneously as they landed on each cloth exposed on a large black cloth background that was wrapped around his legs, his presence alone may have acted as a determining factor for the flies' landing responses on certain cloths. The black background itself presented an attractive target. Furthermore, the method of ascertaining the total numbers of simuliids landing on each cloth may have led to erroneous results, especially at high fly densities. In spite of these few minor irregularities it is interesting to note that the present study showed relatively similar results for *S. venustum* at Pickavance Creek with a preponderance of this species collected on 'purple-blue' silhouettes during both seasons tested. *Simulium vittatum* also showed a strong preference to 'purple-blue', with black and red being the next most frequented colors. Fredeen (1961) devised animal silhouettes to study

the attacking behavior of *S. arcticum*, a serious pest of livestock in Saskatchewan. Using a dark blue denim cloth to cover the silhouettes, he was able to attract this species in 'representative' numbers more so than collections obtained in light traps. Similarly, Peschken (1960) exposed spheres painted blue, black, red, yellow and green, which were coated with Tanglefoot. The results indicated that simuliid species were attracted to the black, blue and red spheres 'very significantly' more than to yellow or green ones. Peschken's results, however, showed total numbers of black-flies with no reference to any particular species preference or difference in species responses to the various colored objects. He is vague as to just what species are, in fact, involved in each of the tests but his seasonal abundance and activity results indicated 'large numbers' of *S. decorum*, *S. vittatum*, *S. venustum* and several specimens of *S. hunteri*, while *Cnephia dacotensis*, a non-blood sucking simuliid, was most abundant at one of his sites. From the present study it appears that *Simulium* spp. in general may elicit specific responses to certain colors or combinations of these colors in a similar fashion and that *S. venustum* shows a very clear pattern of responses to 'purple-blue' colored stimuli, although Davies (1961) found red and blue equally selected by black-flies (mainly *S. venustum*). It is obscure as to which color best attracts *S. vittatum* for it maybe that all three colors are equally effective for this species. On the other hand, the *P. mixtum* complex and *C. mutata* populations are attracted consistently and in abundance to black and red respectively, responding to blue in very low numbers. It is believed this data represents the first responses of these groups of

Simuliidae to color and it may well be an indication of generic differences in the appropriate receptor system within the family.

Lowther and Wood (1964) secondarily ascribed a color stimulus or color pattern attending *S. euryadminiculum* locating its host, the common loon, with a 'very dark green head and neck marked posteriorly by the white "necklace"' (p. 913). In this respect Bennett *et al.*, (1972) found that this species, when presented with two and three dimensional 'silhouettes,' preferred white in combination with two dimensional forms but that black was the most attractive color when used with a three dimensional form. The former appeared to be related to a resting response while the latter suggested the fly was responding to a blood-meal, thus substantiating the 'dark-green' response postulated by Lowther and Wood (1964).

Black garment materials were found to be seven to nine times more attractive to *Simulium galeratum* Edw. and *Gnus chołodkovskii* Rubz. than white or green (Fedder and Alekseyev, 1965), while red and blue was next to black with approximately equivalent numbers. It is of interest that the determination of the comparative attractiveness of these colored materials to black-flies of the USSR, consisted mainly of a subgenus of *Simulium*, *Gnus chołodkovsky* (90%). Fedder and Alekseyev (1965) suggested that greenish-yellow materials were "the least attractive to black-flies and that clothing of this color acts as a camouflage and protects a person from attack" (p. 162). They made no reference to the total luminous reflectance of each garment but explained that the variation in results obtained for two green colors was due to the different reactions of black-flies to

different tones of the same color. In addition to the simuliid responses, Fedder and Alekseyev tested two other groups of blood-sucking Diptera and found black to be the most attractive to the biting midges of the genus *Culicoides* (Heleidae) and mosquitoes of the genus *Aedes* (Culicidae). Brown (1951a) also found that *Aedes* mosquitoes were attracted more so to dark colors than light and Brown (1952) and Smart and Brown (1956) observed that dark-skinned Caucasians were more attractive than light, while Negroes were highly attractive. Glossy black spheres are highly attractive to the Tabanidae (Bracken, 1960; Bracken *et al.*, 1962; Hanec and Bracken, 1962; Bracken and Thorsteinson, 1965; Thorsteinson *et al.*, 1966) while Granger (1970) found that dark brown as well as black were the most attractive colors for *Tabanus nigrovittatus* Macquart and Snoddy (1970) found that his red and black balloons, coated with Tanglefoot were 45 times more attractive to *Chrysops niger tayleri* Phillip than adult human males.

The present results reveal quite a definite preference among species of Simuliidae for certain colors. The knowledge and correct identification of each species has obvious importance in control, especially for those haematophagous Diptera capable of transmitting various diseases. Identification of each species involved would undoubtedly provide necessary information for potential use in the design of traps and trap color as efficient sampling methods for haematophagous populations. On these grounds it should be noted that any generalization as to the responses or attraction of 'black-flies' to color in general is suspect.

Davies (1951, 1961) established that for black-flies the

frequency of landing on color decreased with increased intensity of the light reflected from each cloth surface. He (Davies, 1951) computed a "landing percentage" and showed that the cloths with the smallest landing percentage had the highest "reflectance ratios," viz., few simuliids landed when the total intensity of the light reflected from the cloths was high but that those dark cloths with the highest ultra-violet reflectance were the most attractive. Golini (1970) also observed that all simuliids landed on a silhouette with a low reflectance of light. The present work shows a similar pattern of responses except that in the 1971 season, black, which has an extremely low luminous reflectance, attracted fewer simuliids than either the red or 'purple-blue' color, each with a higher percentage of total reflected light. This may be due to the fact that larger populations of the *S. venustum* complex and *S. vittatum* were present for that season, both species of which are attracted to 'purple-blue' and red colors in total numbers than that for black. Although the *P. mixtum* complex and *C. mutata* prefer black to other colors, the collections obtained in the same period reveal a preponderance of both species to red which appears to be a preferred subordinate choice. Inclusive of these facts with consideration of that period of the season when this particular experiment was exposed, it was possible that the June 15 collection date coincided with a peak in the populations of *P. mixtum* complex, *C. mutata* and *S. venustum* (first generation) and the subsequent July 17 test period with that of *S. vittatum* and large numbers of *S. venustum* (second generation), (Fig. 13b). Accordingly, it may account for the majority of simuliids

on the 'purple-blue' silhouettes. It was realized that these results probably indicate an exaggerated species response to a particular color independent of its total percentage of reflected light, and hence the important effect color has on the near-orientation and landing behavior of Simuliidae. It would seem reasonable to postulate that these flies locate a 'host' at long-range by means of the host's chemical odors and/or carbon dioxide, after which they use various visual stimuli such as color to direct near-orientation and landing behavior.

For *Aedes* mosquitoes Brown (1954, 1966) observed that attractiveness varied inversely with cloth reflectivity or brightness, especially between the limits of 625 m μ . and 475 m μ wavelengths, but that textures of the cloths obscured this generalization. Spectro-sensitivity data presented by Peschken (1960) demonstrated that black flies were mainly sensitive to wavelengths 380-550 m μ . Davies (1951) established that blue was the most attractive to *S. venustum* as were colors reflecting the highest intensity between 400-660 m μ . It is known that insects are probably capable of perceiving a very large spectral range approximating 240-700 m μ . although Hocking (1964) points out that the ozone layer in the atmosphere is responsible for the limitation of the solar spectrum at the ultra-violet end at about 0.29 microns at the surface of the earth. Thus, although an insect can perceive ultra-violet radiations, it is probable that it is limited to the range 300-670 m μ . The most attractive colors used in this study had a spectral peak of 450 m μ . ('purple-blue') and a spectral range of 580-700 m μ . (red) (Table I; Fig. 21), spectral data

similar to those used by these researchers. Similarly, the Kodak Wrattin filters had spectral dominant wavelengths of 615.3 μ . (red tricolor) and 631.6 μ . (deep red tricolor) (Table II). Due to the fact that long wavelengths (i.e., red filters) require higher intensities of light to standardize wavelength emission with those of shorter wavelength (viz., blue tricolor, 463.8 μ .), the flies may have responded to the dimly lit red filters instead of the actual monochromatic wavelength emitted. Tabanidae, on the other hand, seem to prefer black glossy objects rather than matte black ones in the field but since black-flies are not active during bright sunlight, Thorsteinson *et al.*, (1965) figured such a trap would be inferior for the collections of these insects.

The basic method of examining color "vision" of insects and indeed the entire animal kingdom has been to determine whether the experimental subject can discriminate between two visual stimuli of different wavelengths or wavelength compositions, independent of their brightness or intensities. Mazokhin-Porshnyakov (1969) advises any study of animal vision to use the term "radiation" instead of "color," because "we do not know how the animals perceive colors and, secondly, because color does not fully characterize the physical composition of a radiation" (p. 146). For example, certain colors may appear identical and yet contain different spectral compositions, viz., a yellow light can be monochromatic or composed of green and red radiations.

An animal's reaction to radiation or color and the evidence for color vision has been principally derived from two sources: (1)

observation of behavior ("direct") and (2) by electrophysiological observations on the functional changes occurring in the eyes, compound or otherwise ("indirect"). The present study involves the "direct" behavioral approach and no attempt is made to derive a relationship between the spontaneous reaction of the insect to colored silhouettes composed of several radiations and that of true color vision for the same insect. It is assumed that the simuliid's behavioral response toward color are part of a chain of processes initiated with the reception of carbon dioxide. The experimental protocols herein remove the individual fly from the population following its response to a colored, inanimate silhouette. Interpretation of the response of the black-flies to colored silhouettes is therefore based on a statistical analysis of the behavior of samples of the populations. Approximate information on the visual responses of Simuliidae was obtained. A clear understanding of some of the above inherent problems is essential before the responses of the simuliids are interpreted too broadly.

Honeybees, *Apis* spp. (Daumer, 1956) and bumblebees, *Bombus* spp. (Mazokhin-Porshnyakov, 1969) possess a complex system of color vision with three receptor groups known as trichromatic vision. The trichromatic visual color system is usually expressed in the three parameters, radiation (hue or wavelength), intensity (value or brightness) and chroma (color saturation or purity), which characterize the state of excitation of the three receptor systems known in bees. Several dipterans, such as *Calliphora erythrocephala* and *Musca domestica*, have two receptor groups or dichromatic vision (Mazokhin-Porshnyakov, 1969). Possibly other dipterans, such as Simuliidae,

possess a similar dichromatic scheme of color vision. It has been shown that Simuliidae are sensitive to radiations within the range 380-660 $m\mu$., but dependent on the total intensities reflected by each of the radiations as well as the mixture of ultra-violet radiation each contains. Color cloths of high chroma were observed to be landed on less frequently (Davies, 1961) than on duller ones with colors of the same radiation and intensity but testing for the importance of chroma was "less extensive." Perhaps the Simuliidae do not perceive chroma as the dichromatic visual system is somewhat inferior to the trichromatic system and fails to discriminate chroma, responding only to variations in radiations and intensity. A test with Munsell standards varying in hue (radiation) but with equal intensities and chroma (Table IIIa) attracted approximately equal numbers of flies. Other tests did not indicate that the frequency of landing decreased with an increase in the intensity of the light reflected from the standards as Davies (1961) had found. The Munsell blue standard (Table IIIa) appears to be attractive to certain species of Simuliidae and it is possible that variation within this hue (radiation) would have no measurable effect on the frequency of landing. Further tests showed that the landing rate on the colored standard 5B 6/6 was similar to that on a neutral (achromatic) standard having an identical intensity (value) attribute, (viz., N 6/6, Table IIIb). If it could be clearly shown that the simuliids occur in significant numbers on various radiations independent of its intensity, as attempted here (Table IIIb), then the effects of certain radiations in directing a simuliid to a stimulus surface could be more intelligently discussed.

Standardization of experimental protocols through such systems as the Munsell color notation is essential before the visual responses of these or other animals can be adequately assessed.

Causation may be denoted by the efferent systems and afferent mechanisms of an animal's behavior. Behavioral elements of different degrees of complexity can be released, maintained, terminated, inhibited, primed, and directed by specific external situations (Baerends, 1959). This situation involves efferent systems of the organism. However, it has been recognized (Baerends, 1959) that an object may release a complex of behavioral elements that are superimposed on or substituted for one another and that in this case each element may receive its own releasing and directing stimuli from the object (afferent mechanisms). Simuliids as well as other biting-flies have often been observed to display different behavioral elements when in a certain physiological state, for example a female seeking an ovipositing site. Peschkin (1960) and Peschken and Thorsteinson (1965) found that black-flies laid significantly more eggs on yellow and green-colored sticks while avoiding black and blue ones. Similarly Golini (1970) showed a contrast between the behavior of host-seeking black-flies and those ovipositing. "This maximum response of simuliids for the blue-end of the spectrum apparently shifted during the oviposition state toward the longer wave-lengths of the yellow spectral region" (p. 190). He suggested that a color preference was shown to be related to the ratio of the quantity of reflected light from each color in the 450-700 μ . and 325-450 μ . (ultra-violet-blue) ranges of the spectrum and that the degree of response to reflected light of certain radiations

depends on an "interplay of the longer and the shorter wave-lengths of the visible spectrum" (p. 190). It is interesting in this context that *A. aegypti* mosquitoes were reported to be rather insensitive to long (red) wavelengths and responded to a peak, 550 m μ . in the yellow-green range when ovipositing (Snow, 1971). It has been shown previously that host-seeking mammalophilic simuliids do not respond in any significant numbers to yellow which had a similar peak 560 m μ . (Table I, Fig. 21). The coating of Tanglefoot does not provide an opportunity for the flies to seek a subsequent choice of "host" surface stimuli and may explain why there were some specimens collected on this colored silhouette. The insect's failure to respond to certain conditions in the environment however, does not mean that it is incapable of perceiving similar stimuli under opposing circumstances. Tinbergen (1969) illustrates conclusively that "the strict dependence of an innate reaction on a certain set of sign stimuli leads to the conclusion that there must be a special neuro-sensory mechanism that releases the reaction and is responsible for its selective susceptibility to such a very special combination of sign stimuli" (p.42) and that "different reactions of the same animal have different releasing mechanisms" (p.43). Bennett *et al.*, (1972) suggested *S. euryadimniculum*'s preferred response to dark-colored vertical silhouettes was a host-seeking response while that of white horizontal silhouettes indicated a rest response. He explained that sun-bleached rocks presented an almost white resting silhouette to these lacustrine flies in nature.

It is quite likely that radiations such as yellow and

green etc., blend with similar colors of the fly's natural surroundings rendering such colored silhouettes or "hosts" an almost imperceptible target or "camouflage." Males of biting-flies typically orient or gather in mating swarms above objects contrasted against the environment by their relative brightness or darkness. Bässler (1958) showed the importance of contrast for male *Culex pipiens* mosquitoes in the formation of swarms above sheets of white paper laid out in a meadow. Several investigators have shown the importance of background illumination for ovipositing haematophagous Diptera (Williams, 1962; Golini, 1970) while Christophers (1960) attracted ovipositing mosquitoes to black papers contrasted on a white ground or other highly reflecting surface such as a mirror or glass. It would be interesting in this respect to test the responses of Simuliidae to vertical yellow and green silhouettes exposed against a "purple-blue" or other attractive dark reflecting background radiation, to determine if it would have an opposite but stimulating effect. In fact, it is conceivable that "purple-blue", black and red silhouettes in the near vicinity of the yellow and white ones were responsible for enhancing the number of flies landing on "non-attractive" vertical silhouettes such as the latter, i.e., attractive radiations appear to enhance inferior radiations for host-seeking Simuliidae. Even among "attractive" radiations, each may enhance the other's "catching" capacity and help to explain the inconsistencies in the responses of *S. vittatum* (Fig. 14, 18). However, each species response must be regarded individually.

The role of silhouette shape in the visual near-orientation
and landing behavior of mammalophilic Simuliidae

Following two seasons of experimentation it was quite evident that the existing silhouette shapes were not significantly different in their individual contours to elicit specific responses among the various species of Simuliidae tested. Completion of the first summer's testing indicated that the 'disc' silhouette was preferred above the other shapes during each of the exposure periods, although statistical analyses had not been performed. As a result, this shape was used in subsequent experimentation and large numbers of simuliids were trapped on it. It was then realized that any of the silhouettes could have been employed for the same purpose. In his thesis, Peschken (1960) concluded that black-flies were more attracted by solid patterns and that different patterns could only be recognized by their "degree of brokenness." He defined solid patterns as triangles, circles and squares, etc., while the "broken outlines" presented a greater optomotor stimulus than solid figures and hence the black-flies could visually discriminate between these two classes of shapes and that "increased optomotor stimulus is inversely correlated to attraction" (p. 68). The targets used in this study represent Peschken's "solid" figures and possibly the simuliids could not "significantly" discriminate between them. The use of variously-shaped silhouettes did, however, indicate that various parts characteristic for each shape were landed on consistently. In most cases there was a preponderance of flies on the corners of the squares and rectangles, the vertices of the triangles and the points of the stars, whereas flies

on the disc were more or less randomly distributed.

Studying the attacking behavior of black-flies, Wenk (1963) found that certain female *Wilhelmia* spp. preferred projecting parts (viz., ears of horses and cows) of larger, moving objects when seeking a host for a blood meal. He observed that some ornithophilic species preferred small avian 'targets' which moved and had projecting parts. Peschken and Thorsteinson, (1965) indicated similar effects of physical elements characteristic of the host on the attacking behavior of Simuliidae. Convex plane figures such as squares and discs were significantly more attractive than concave figures of X and Y. Again, Fallis *et al.*, (1967) collected more simuliids from the end portions of horizontal "sticky" paper cylinders than on the mid-portions. Similarly, Bennett *et al.*, (1972) noted that *S. euryadminiculum* were more numerous on the most prominent portion of their "head-neck" decoy silhouettes. These results would seem to suggest that the various "points of convergence" of silhouettes are favored landing sites for simuliids seeking a blood meal. If one considers the relative ease a black-fly such as the *Wilhelmia* spp. could obtain a blood meal from the ear of a horse or cow, as compared to some other tougher hide of the body, then such attacking behavior could be appreciated. It might well reflect an evolutionary plasticity of responses of blood-sucking simuliids.

The role of silhouette size and surface area on the near-orientation and landing behavior of mammalophilic Simuliidae

Quantitative estimates are required of the importance and role of physical parameters of the host in guiding the flight paths of simuliids in host-seeking behavior. Aside from color and contour

reactions, the Simuliidae have been reported responding to certain hosts dependent on the particular area of host exposed to them (Fredeen, 1961). Similarly, for certain culicids, Downe (1960) found their attraction to various avian and mammalian hosts to be influenced more by the size or surface area of the host than by other factors.

Many mammalophilic species of Simuliidae such as *P. mixtum*, *P. fuscum*, *C. mutata*, *S. venustum* and *S. vittatum* have been commonly observed attracted to vehicles and humans, feeding in abundance on various large mammals in the same vicinity where avian hosts were exposed (Anderson and Defoliart, 1961). These black-flies did not feed and were rarely attracted to the birds. Similarly, they were rarely attracted to various small mammals such as skunks, squirrels and domestic white rabbits and on the same days when these hosts were exposed, a number of these same simuliids were taken feeding on man, cattle and horses in the immediate areas. It was concluded that these mammalophilic simuliids seek out and feed on the larger mammals in preference to small animals. It was shown in the present study that more mammalophilic Simuliidae (mainly *S. venustum*) were collected on the black disc silhouette with the larger area exposed. These results indicate that these particular sizes of targets were of significance in attracting the mammalophilic simuliids of the Avalon Peninsula and that their attraction was in approximate proportion to their size (viz., 1:1.8:2.7, Fig. 20). It has been suggested by Pickavance *et al.*, (1970) that the general paucity of both wild and domestic large mammals on the Avalon Peninsula may have forced simuliid species, such as *C. mutata*, to seek unexpected hosts. They collected a single engorged female from

a duck bait at Pickavance Creek and *S. venustum* was taken from both wild rabbit and duck baits but they were not certain whether the flies had fed on the duckling. This evidence suggests that in the absence of large mammals, the black-flies are attracted to small mammals. Realization of an optimal size of target for simuliids of the Avalon Peninsula may be a field study worth extensive investigation. For example, Bracken and Thorsteinson (1965) found the attractancy of adult tabanids was not increased by enlarging the diameter of decoy silhouettes beyond 30.7 cm. The original disc diameter used in all experiments throughout both field seasons in this study was comparatively as good in attracting simuliids as the other disc diameters employed in specific studies.

The importance of vision and olfaction in the near-orientation and landing behavior of mammalophilic Simuliidae

Simulium venustum was demonstrated to have a sensory capacity to discriminate between traps emitting carbon dioxide at different rates (Smith, 1966) even when the traps are very close together. Olfaction differs from sight and hearing by virtue of it being a non-directional sense but the mechanism whereby the odor guides the insect so precisely to its goal is as yet unknown (Wright, 1958). Theoretically, it has been considered possible for an insect to sense an odor through the frequency with which it encounters the odor cloud filaments or lamellae which are carried in a twisting fashion down-wind from the odor source. In view of the filamentous structure of an odor cloud, the insect, while it is in free flight, probably searches by flying a series of long, "zig-zag" paths and when it enters the cloud its

tendency to turn is inhibited as long as the interval between regions of high and low odor density tends to decrease (Wright, 1958). Daykin *et al.*, (1965) demonstrated that mosquitoes change their behavior when they contact a neutral zone to effect orientation once more into a stimulating region of an odor substance. It remains doubtful as to the extent an odor-cloud can produce by itself a reasonable accurate homing reflex in the flying insect. It is known that insects do use vision as a means of up-wind guidance and that the recognition of an olfactory guidance system does not preclude reinforcement by other senses such as vision. Wensler (1972) did observe, however, that mosquitoes rarely arrived at a control dish (without an odor-producing substance) which was exposed in a position comparable to the "odorous" dish. She suggested that insects do not land at random (a reaction Thorsteinson and Brust (1962) did favor) and that mosquitoes landed at random at visually similar stimuli and not by orientation to the odor. As Tinbergen (1969) pointed out, "an animal responds 'blindly' to only part of the total environment situation and neglects other parts, although its sense organs are perfectly able to receive them (and probably do perceive them), and although they may seem to be less important to the human observer, than the stimulus to which it does not react" (pp. 25-26). The possibility that combined guidance clues are ever present does exist and each step in the total hierarchy of responses attendant on the insect locating its goal may be defined in terms of the sensory makeup through which they are mediated. A study of the relationship between these factors and the degree to which each sensory mode is employed by the insect during orientation to a host

may be estimated as a function of the distance in which each factor is involved. This type of approach may be the only suitable means for field studies of this problem. Thus it could then be possible to suggest the sequence in which the various stimuli are presented to the insect.

Gillies and Wilkes (1969) proposed that orientation of mosquitoes to warm-blooded hosts could be regarded as a sequence of stimulus response situations experienced as they flew up the "host-stream," and described this "plume" and "cloud" in terms of zones corresponding to the ranges at which successive host stimuli were first encountered by the approaching insect. The various factors were classified into close-range, middle-range and long-range zones. Close-range factors comprised visual stimuli, especially in the case of diurnal mosquito species, together with warm, moist convection air currents; carbon dioxide at middle-range and host specific odor at long range. The influence of these factors was thought to be cumulative, for instance the presence of host odors may modify the responses to carbon dioxide, etc. Their hypothesis was supported by the fact that females of *A. melas* could converge toward two bait calves from at least a 40 yd. distance, whereas when the equivalent output of carbon dioxide was substituted for the animals, convergence was only initiated from between 20 and 40 yds. Therefore, they concluded, olfactory cues were responsible for the long-range orientation as indicated by *A. melas*. *Culex* spp., on the other hand, were orienting to both calf baits and carbon dioxide from 20 - 40 yds. such that similar long-range effects for these species of *Culex* was lacking.

The Simuliidae are typically diurnal insects and undoubtedly use visual cues to mediate orientation relatively near the host. The extent to which they use their visual capacity is not known. Realization that specific host odors are only known for one species of Simuliidae, *S. euryadminiculum*, it is reasonable to assume that medium-range orientation to a host is mainly gained through up-wind orientation in a carbon dioxide, wind-dispersed cloud cone for most species of Simuliidae. Smith (1966) suspected that the combination of bird extracts with carbon dioxide increased the number of flies coming to the baits as compared to the number coming to carbon dioxide alone. The presence of host odors seems to fortify the response to carbon dioxide and subsequently the combination of the chemical stimuli, or either alone, would appear to enhance the response to specific factors required by the fly to facilitate landing behavior. Smith also considered vision of considerable importance in the near-orientation of simuliids but he observed the reluctance of many flies to land and suggested that "the visual requirements are specific, or that other factors may mediate landing responses" (p. 121). It is conceivable that simuliids use vision at medium range but this is mainly as anemotaxis i.e., upwind directed flight that maintains a constant passage of background images over the insect's eyes from front to back. Kennedy and Moorhouse (1969) concluded on locust responses to wind-borne grass odors that positive anemotaxis was a component of both food-finding and evasive behavior. They agreed with the classical hypothesis that odor both activates the insects and switches on their orientation responses to directional cues provided by the wind itself. The simuliids decided use of vision as

a means of near-orientation and mediated landing behavior is one of a directed nature and an estimation of this 'near' distance from the host is required.

Results on the approach and landing behavior of simuliids to silhouettes comprising various radiations clearly demonstrates that black-flies do decidedly use vision independent of carbon dioxide olfactory stimuli in mediating 'near' and landing processes. Results from figure 17 indicate the various distances from a carbon dioxide source that simuliids are locating and orienting visually to the various silhouette radiations. It is shown that the radiation is rather important in directing this response and appears specific for certain species. It was observed previously that the 'blue' (purple-blue) collected larger numbers of simuliids with black and red next but that few landed on either white or yellow radiations. It was also suggested that the presence of the former radiations enhanced the response to the latter ones. It was indicated that flies were orienting and landing on 'blue' silhouettes as much as 10 m. downwind from the source and the numbers generally increased toward the source, the numbers on 'blue', four metres from the source, being considerably higher than those on 'blue' 10 m. away from the source of carbon dioxide. A reasonable proportion of the total catch was trapped on silhouettes located at distances up to 7.75 m. from the carbon dioxide source, but at distances beyond this the numbers of flies were sharply reduced. Although the main concern of the experiment was not to test the functional orientation distances for these insects, it was considered a reasonable indicator of this

behavior. Whereas only three exposure periods were used to demonstrate the fly's discriminating capacity for color objects, it is likely that certain silhouettes occurred more frequently nearer the carbon dioxide source than others, despite the fact that they were positioned according to tables of random numbers. It is suggested that this defect may be responsible for some exaggerated responses to certain silhouettes on the part of the flies. Golini (1970) separated collecting "quadrants" at distances down wind of one, 10 and 15 feet from the carbon dioxide source which was located at the center of the one foot "quadrant." The relative density of flies showed that a negligible number was collected at 10 and 15 feet from the carbon dioxide source as compared to a distance of one foot. Thus Golini was doubtful that upwind orientation of *S. venustum* could occur with any precision at greater distances than 15 feet from the source of the gas. His "quadrants", however, did not present a stimulating silhouette, were colorless and the carbon dioxide source obviously created a 'bias' catch at the one foot quadrant. It is important in this respect that Fallis *et al.*, (1967) observed *S. venustum* to move toward and alight in "large" numbers on objects near which carbon dioxide was escaping regardless of the rate of carbon dioxide emission. In the present study, the carbon dioxide source was placed four m. up-wind from the nearest silhouette. It was observed though that the number of flies tapered off at distances four m. and beyond. Whereas it was shown that each simuliid species prefers certain silhouette radiations, one may indirectly hint at what distances carbon dioxide is effective in stimulating some of these species to its source. For instance, in

1971 'blue' attracted *S. venustum* in large numbers (Fig. 18) and they may have been orienting up-wind to carbon dioxide from distances approximating 7.75 m. (Fig. 17). Black and red radiations, however, attracted *P. mistum*, *C. mutata* and *S. vittatum* very effectively, thus it is doubtful as to what proportions these species are part of these results but as indicated by the numbers collected on red silhouette radiations, these flies were orienting effectively, at least 7.75 m. away. The numbers on black silhouette radiations show that this stimulus effected orientation possibly as far as 8.75 m. from the carbon dioxide source. It is relevant that Gillies and Wilkes (1970) caught unfed females of *Anopheles melas* Theo. up to distances of 10 yds. from carbon dioxide bait while with other *Anopheles* spp. the range of attraction for the same source was less than 15 yds.

It is conceivable, then, that carbon dioxide cues are responsible for medium-range orientation of at least those species of Simuliidae at Pickavance Creek. The cumulative evidence from the radiation effect of silhouettes placed at various distances down-wind from the carbon dioxide source indicates that at medium-range the radiation compositions of inanimate hosts modifies the carbon dioxide response. The question still remains as to the extent of this interaction of the various radiations and carbon dioxide at near-range orientation.

The field experiments reported here leave little doubt that simuliids have a marked ability to discriminate between targets on the basis of their radiation compositions and largely independent of their individual carbon dioxide emissions. Smith (1966) was able to

demonstrate the ability of simuliids to discriminate between traps very close together but differing only in their carbon dioxide emission rates. On this basis, he concluded that they probably did not rely on vision until they were very close to the host, but he was very careful to point out that the miniature suction traps he employed did not present a "striking silhouette." He provides no estimation of the critical distances when vision and not olfaction serves as the primary orientation cue.

Following completion of the color experiments it was fully realized that some silhouettes were composed of 'attractive radiations' such as the 'blue' (purple-blue) while others were rendered 'non-attractive', comprising a radiation composition in the yellow end of the visible spectrum. This data set the stage for subsequent testing and an estimation of the importance of vision in the near-range attraction of Simuliidae. Similar to Smith's (1966) results, it was observed that 'blue' silhouettes emitting equal concentrations of carbon dioxide collected approximately equal numbers of flies (Table IV) but when one of the 'blue' silhouettes was substituted by a yellow (Table V), very few were captured. It was clear that these insects were discriminating between the silhouettes on the basis of their radiation compositions since both silhouettes emitted equal amounts of carbon dioxide. The next question to be answered was whether the flies could be attracted in equal numbers on the basis of equivalent visual stimulation (Table VI). This proved to be the case for it now was quite clear that if a silhouette provided a 'striking' character it could attract flies in equal numbers irrespective of small

or large quantities of carbon dioxide associated with each. For example, 'blue' silhouettes separated by only 30.5 cm. (centre-to-centre) caught identical numbers of simuliids (viz. 135:134, Table VI), even though carbon dioxide was emitted at 1500 cc/min. at one and only 10 cc/min. at the other. Following the final test (Table VII), there was no reservation as to the visual capacity of simuliids to discriminate between two traps. In fact, in all cases when the larger volumes of carbon dioxide were exposed with the yellow stimulus, flies consistently chose the more visually attractive 'blue' target emitting the smaller quantity.

Smith (1966) initially suggested the possibility that variously-colored traps might effect a dissolution of the discrimination when the traps are separated distances greater than two feet. This appears to be the case, as in Table VII the discrimination coefficient (i.e., the number of flies on 'blue' targets/number of flies on yellow targets) increases until a distance of 120 cm. separating the stimuli is reached after which the coefficient drops considerably. Again, a similar phenomenon is indicative of the results shown in Table V and the discrimination appears to dissolve in the 120 cm.-210 cm. range of distances separating stimuli, i.e., there is a definite selection of stimuli up to this point of increased distances between trap stimuli.

Hocking (1964) reviewed functional morphology of the insect eye and made measurements of some important parameters of the eyes of 28 species. He defined the following:

- (1) Horizontal field of view : "The angle between the most

anteriorly and most posteriorly directed ommatidia ' F_H in Fig.22]...; this is the angle which is bisected in determining the horizontal axial angle" (p. 322), A_H in Fig. 22].

(2) Horizontal binocular field: "The angular overlap between the horizontal fields of view of the left and right eyes, either anteriorly ... or, theoretically, posteriorly" (p. 322), B_H in Fig. 22].

(3) Index of resolving power: "The reciprocal of the ommatidial angle is a valid indicator of resolving power. The mean ommatidial angle in a horizontal plane is given by: F_H divided by the number of ommatida in this plane" (p. 325).

He calculated the insect mean for $B_H = 38^\circ$ and the horizontal index of resolving power to be = 0.52 mm. From this data projected dimensions of simuliid's use of its horizontal binocular visual field is theoretically estimated as a function of distance between two stimuli in a horizontal field and the distance approach used by the fly in the visual near-orientation to the two stimuli, (Fig. 22). Wenk (1965) outlined the swarming behavior of some mammalophilic Simuliidae and observed that the orientation of males toward an optic marker was the last stage in the long-range orientation. Males of *Wilhelmia equina* occupying the uppermost flying position in the swarm, orient vertically so that the marker image is centrally in the median space of its head. Vertical sectionings of the head made by Wenk showed that the overlapping space of both compound eyes was 38° and that the vertical distance from the marker depended on its size but that at a maximum vertical distance of 60 cm. the fly changes its orientation by fixing to a portion of the marker

such as the corner.

Measurements of the angles of overlap of compound eyes of female simuliids have not been attempted here. Male simuliids possess a holoptic condition (meeting along their medial border), while female compound eyes are slightly spaced apart (dichoptic condition) which may permit an overlap space in the horizontal plane of at least 38° . Male insects usually have larger eyes than the females especially the Simuliidae, for example, male simuliids of the genus *Onephia* are reported to have a maximum B_H of 89° (Hocking, 1964). Hocking (1964) calculated the mean horizontal binocular field of vision from measurements of 14 major insect orders and found it to be 38° . Whereas in the case of faceted eyes the resolving power (visual acuity) is determined by the ommatidial angular density, i.e., by the number of ommatidia present in a plane or solid angle, the larger the number of ommatidia included in a given angle or rather the smaller the inter-ommatidial angle, the sharper the vision (Mazokhin-Porshnyakov, 1969). Thus, if one were to relate the near-orientation behavior of simuliids to the existing knowledge of the anatomical field of vision for insects it would function as a rough estimate as to what theoretical distances would be involved in the horizontal up-wind flight to inanimate host objects. Theoretically then, Figure 22 shows that the fly could perceive stimuli separated a distance of 30.5 cm. from a point approximating 45.8 cm. downwind and in that order to distinguish between stimuli separated 120 cm. and 210 cm. it would by virtue of its $B_H = 38^\circ$ have to be at least 173.8 cm. and 302 cm. downwind, respectively, etc. Consideration must of course be given to the resolving power of the

females' compound eyes and it is this parameter which would effect the visual perception of such object stimuli at the various distances of the near-orientation behavior tested. Discrimination coefficients as mentioned for these particular distances between stimuli (Table V and VII) indicate a definite selection by the fly up to this range and from the data presented in Figure 17 it would appear that distances four metres downwind from the carbon dioxide source would be an approximate limiting distance for the simuliids' effective use of vision. It is possible that in this region of near-orientation the visual receptors begin to direct the insect's flight path to its goal.

The results in Tables IV-VII are mainly indicative of the responses of *S. venustum* but *S. vittatum* did compose some of the collections. Golini (1970) observed that both carbon dioxide alone and in combination with a visual stimulus were found effective in attracting *S. venustum*. He demonstrated that the combined influence of olfaction and vision caught the greater number (almost double) of flies when compared to the number attracted to carbon dioxide (olfaction) alone. Golini did show, however, that a white trap collected significantly fewer flies than a black trap at equal rates of carbon dioxide emission. He also observed that the discrimination ratio between the black and the white trap seemed to decrease noticeably as the carbon dioxide output beside the white trap was increased and a discrimination ratio = 1 for *P. fuscum* was reached at the white trap when the carbon dioxide emission was three to four times that of the black trap. He designed these experiments to show the relative importance of reflected light and carbon dioxide emission. It is conceivable

that silhouettes comprising attractive radiation compositions are, in fact, more important in directing near-orientation and landing behaviour in simuliids as the present results have indicated.

On the basis of these observations and data it is herein considered to adduce and expand Stage II of Smith's (1966) behavioral hierarchy for the Simuliidae to include two further orientation mechanisms which appear to be involved in the up-wind orientation of simuliids.

Stage II. Up-wind Orientation

a.) Long-range Orientation. It is proposed that host specific odors such as those known for *S. euryadminiculium* releases, in this unique species, a host-directed, up-wind response. It is probable that this special olfactory cue acts as a releaser at distances further than that known for carbon dioxide alone but that carbon dioxide modifies this response at a certain distance from the host.

b.) Medium-range Orientation. The distance whereby carbon dioxide begins to direct most simuliids including *S. euryadminiculium* to a host and most likely provides the main guide-posts in the directed orientation in these insects. Vision, in as much as it is involved in anemotactic orientation, may also be functional. The distance downwind from a host where more "directed" visual responses occur should then be regarded as near-orientation behaviour. It is possible that vision modifies this stage.

Stage III. Near Orientation

This area involves stimulation due to the physical makeup of the host, such as color, movement, shape and area, etc., at distances not exceeding four meters downwind from the host. The extent of carbon dioxide modification at this level of orientation is largely dependent on the 'attractive quality' or 'prominent features' of the host and involves visually-directed behavior.

SUMMARY

1. Records of the seasonal succession and seasonal range of several species of adult mammalophilic Simuliidae of insular Newfoundland are presented. Their patterns of emergence are discussed in relation to Simuliidae in Ontario streams.
2. Collections of Simuliidae on variously colored-shaped two dimensional silhouettes indicated particular patterns of 'preferred' near-orientation and landing responses for certain species.
 - (a) The *Prosimulium mixtum* complex, and *Cnephia mutata* were attracted significantly more to black colored silhouettes.
 - (b) The *Simulium venustum* complex was attracted significantly more to 'blue' colored silhouettes.
 - (c) *Simulium vittatum* was attracted significantly more to both red and black silhouettes.The color responses of these simuliids are discussed as an indication of generic differences in the appropriate receptor system within the family.
3. The most attractive colors for all species were black, 'blue' and red respectively, indicating that the silhouettes reflecting the least amount of light were the most attractive. Depending on the species composition of the simuliid population, however, this generalization tended to become obscure.
4. Yellow was the least attractive color to all species. The physical makeup of stimulus attraction directing the behavior of the insect

is discussed in relation to specific external and internal conditions of the fly's environment required to release various opposing behavioral elements.

5. The points of convergence of the silhouette shapes attracted large numbers of flies. The individual contours were equally attractive and this is discussed with reference to greater 'optomotor' stimuli.
6. An attempt was made in the field at projecting a light source through various spectral filters of known wavelength emission. The spectral range 380 - 660 m μ . and its attraction to Simuliidae especially *S. venustum* is mentioned.
7. Standardized tests were conducted on the near-orientation and landing responses of Simuliidae using the Munsell Color Company system of color notation. The results are discussed with reference to a dichromatic color-visual system of the Diptera.
8. Exposition of silhouettes varying increasingly in proportionate size and surface area indicated a proportionate increase in the numbers of simuliids landing. Their responses are discussed with special mention to the paucity of large mammals on the Avalon Peninsula.
9. The responses of Simuliidae to 'attractive' and 'non-attractive' silhouettes and controlled release of gaseous carbon dioxide indicated a marked ability of the flies to visually discriminate the targets largely on the basis of their radiation compositions

and independent of the concentration of CO_2 or olfaction.

10. The total hierarchy of responses attendant on the insect orienting and locating a host is discussed in terms of the sensory make-up through which they are mediated. Hence, long-range, medium-range and near-orientation mechanisms are presented for the Simuliidae. Estimations of the distances down wind from a host in which each behavioral element is involved is presented.

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Figure 1.

Aerial view of Pickavance Creek (black line) showing the experimental site (black box) which is seen in the inserted color photograph. Note that Pickavance Creek is adjacent to the site.

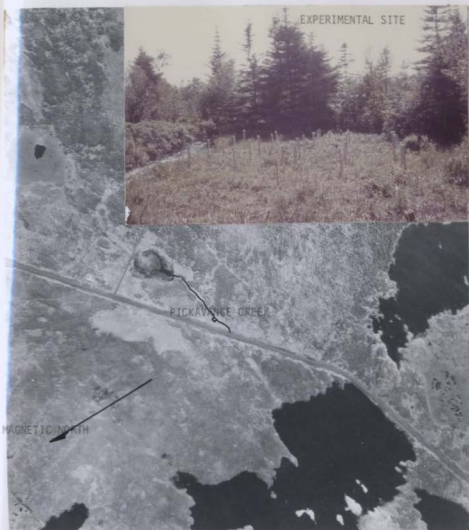




Figure 2.

Simple two-dimensional geometric forms, variously-colored, exposed at Pickavance Creek, experimental site on rotating display platforms of the 1970 design.

- a. rectangle
- b. triangle.



Figure 2.

Simple two-dimensional geometric forms, variously-colored, exposed at Pickavance Creek, experimental site on rotating display platforms of the 1970 design.

c. square.



Figure 3.

Rotating display platform of the 1971 design. Note that an exposure period for the size-discrimination experiment is in progress using black disc-shaped, two-dimensional silhouettes.



Figure 4.

Red, square-shaped, two-dimensional silhouette covered with saran wrap following completion of an exposure period. Note the preponderance of simuliids on the corners or "points of convergence" and method of silhouette attachment.



Figure 5.

Meteorological instruments. Sensitive cup anemometer (left) and wind indicator (right).



Figure 6.

Twenty-five variously-colored, simple, two-dimensional silhouettes secured on stakes comprising an experimental grid site at Pickavance Creek.



Figure 7.

Testing the landing responses of Simuliidae to a horizontal display of 25 stimulus cards at the experimental site, Pickavance Creek.



Figure 8.

- a. Rear view of projection booth display Kodak Wrattin Gelatin Filters. Note slide projector.
- b. Front view of projection booth showing screen port and filters. Note CO₂ outlet in front of screen port.



Figure 9.

Testing various combinations of chroma (color) and achroma (neutral) Munsell Standards. On the left, hue varied with value and chroma attributes constant, while the display on the right exposes six achroma standards in combination with a chroma standard. Note polyethylene coverings on the Munsell stimuli.



Figure 10.

Pickavance Creek experimental site showing an exposure period (background) of two display platforms containing disc-shaped stimuli varying in proportionate areas (1:2:4).



Figure 11.

- Testing the ability of Simuliidae to discriminate between stimuli on the basis of the radiation composition and CO_2 concentration associated with each.
- Clothesline strung N-S over grid site, Pickavance Creek.
 - Two blue disc silhouettes and their CO_2 outlets (during the exposure period variously controlled CO_2 concentrations were emitted).



Figure 11.

Testing the ability of Simuliidae to discriminate between stimuli on the basis of the radiation composition and CO₂ concentration associated with each.

- c. Side view of same. Note CO₂ outlets tied to clothesline and centred at the target disc.



Figure 12.

Format of experiment testing Simuliids' ability to discriminate between two disc-shaped silhouettes on the basis of their radiation compositions and controlled CO_2 emission concentration. N.B. Yellow disc is the non-attractive stimulus.

- a. disc stimuli separated 310 cm. (equal CO_2 emission)
- b. stimuli separated 120 cm. (equal CO_2 emission).



Figure 12.

Format of experiment testing Simuliids' ability to discriminate between two disc-shaped silhouettes on the basis of their radiation compositions and controlled CO₂ emission concentration. N.B. Yellow disc is the non-attractive stimulus.

- c. stimuli separated 91.5 cm. (equal CO₂ emissions)
- d. stimuli separated 30.5 cm. (centre-to-centre) with equal CO₂ emissions.

Figure 13, a (1970), b (1971)

Seasonal succession and seasonal range of adult Simuliidae
at Pickavance Creek, Avalon Peninsula, Newfoundland,
summer 1970, 1971, obtained from
silhouette trap collections.

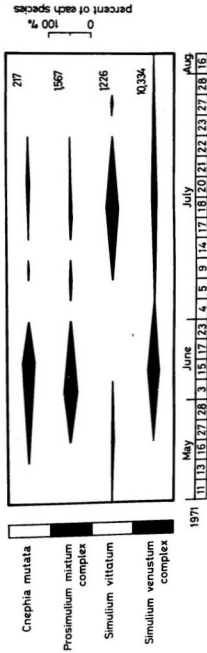
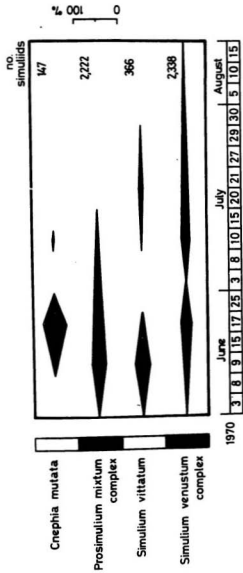
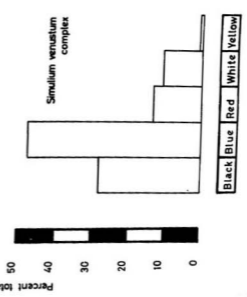
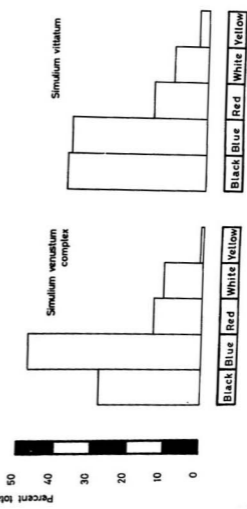
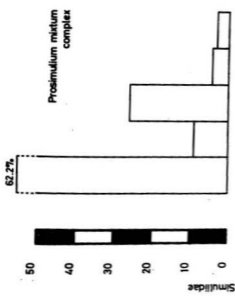
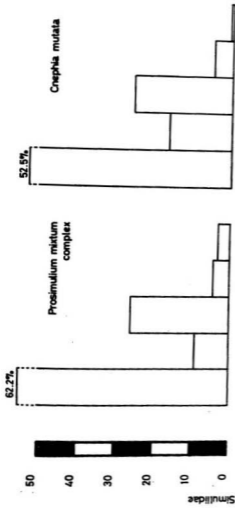


Figure 14.

Landing responses of Simuliidae to variously colored,
two-dimensional silhouettes, summer 1970.



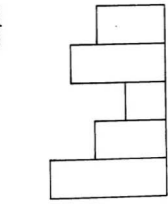
Percent total Simuliidae

Figure 15.

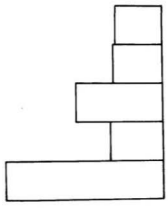
Landing responses of Simuliidae to simple, two-dimensional, vertical, geometric forms, summer 1970.



Prosimulium mixtum complex



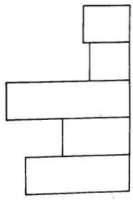
Cnephia mutata



Percent total Simuliidae



Simulium venustum complex



Simulium vittatum

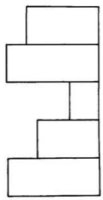


Figure 16.

Landing responses of Simuliidae to variously colored, two-dimensional, vertical, silhouettes as a function of luminous reflectance, summers, 1970 and 1971.

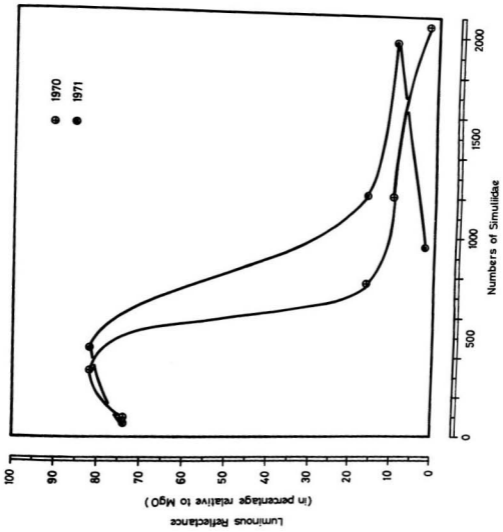


Figure 17.

Landing responses of Simuliidae to vertical, two-dimensional silhouettes as a function of distance from a carbon dioxide source located at zero metres, summer 1971.

no. simuliids = 4,690

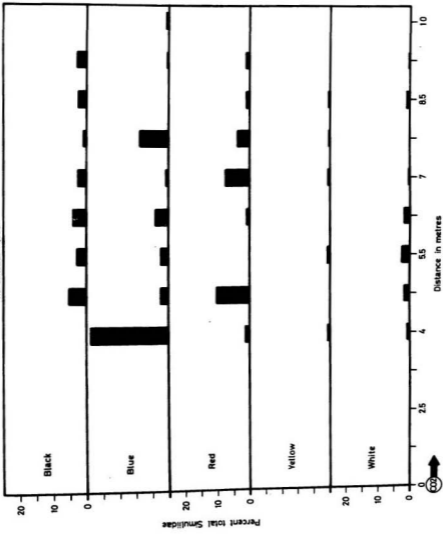


Figure 18.

Landing responses of Simuliidae to variously colored,
two-dimensional, vertical silhouettes, summer 1971.



50

40

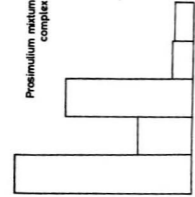
30

20

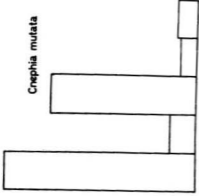
10

0

Prosimulium mixtum
complex



Crepiphia mutata



Percent total Simuliidae



50

40

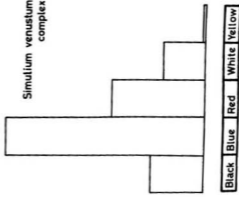
30

20

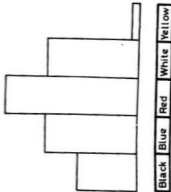
10

0

Simulium venustum
complex



Simulium vittatum

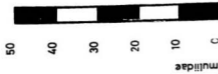


Black Blue Red White Yellow

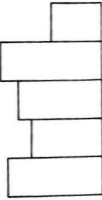
Black Blue Red White Yellow

Figure 19.

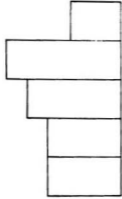
Landing responses of Simuliidae to simple, two-dimensional
vertical, geometric forms, summer 1971.



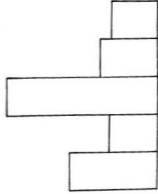
Prosimulium mixtum
complex



Cnephia mutata



Simulium venustum
complex



Simulium vittatum

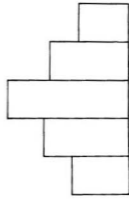


Figure 20.

Landing responses of Simuliidae to two-dimensional, vertical, black, disc-form silhouettes, differing in individual areas exposed.

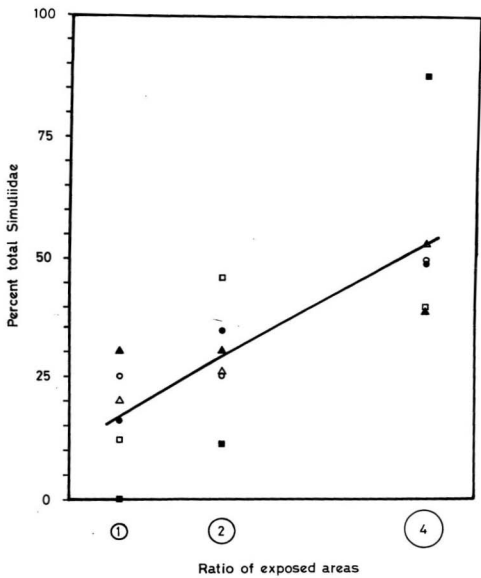


Figure 21.

The variation in intensity with wavelength for each silhouette pigment as determined by comparison with Munsell art materials at the Munsell Color Company laboratory.

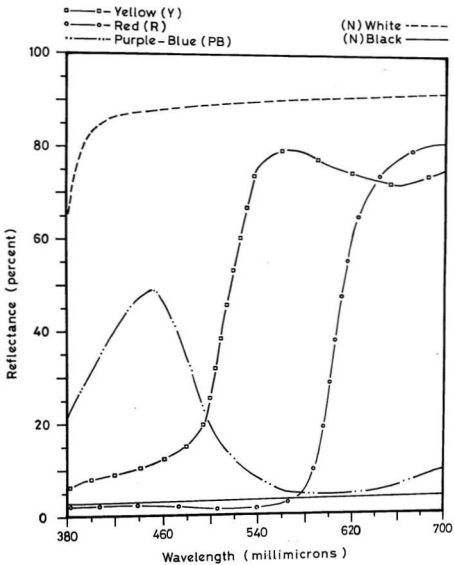
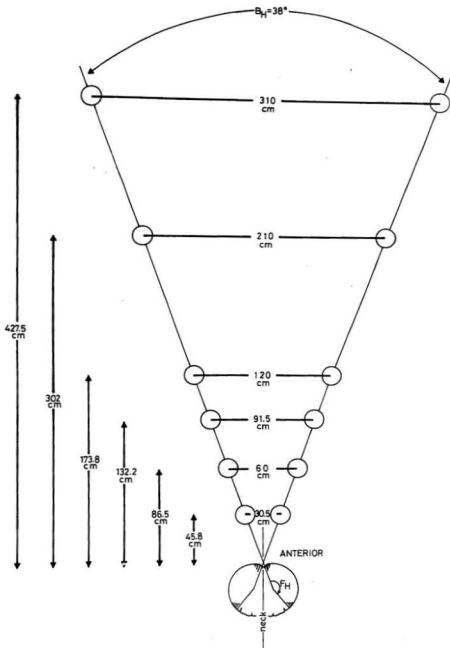


Figure 22.

The theoretical downwind distances involved in near-orientation of mammalophilic Simuliidae, Avalon Peninsula, as a function of distances between disc-silhouettes and the theoretical horizontal angle of binocular vision (insect mean $B_H = 38^\circ$). The diagrammatic saggital section of insect head showing the horizontal field (F_H) was drawn after Hocking, 1964.



APPENDIX I

Analysis of Variance

A three way analysis of variance (m observations . per cell) was conducted using a Wang (700 series) computer to analyse results obtained from 1970 and 1971 summer's field experiments testing the simuliidae near-orientation and landing responses to variously colored-shaped two-dimensional silhouettes. At Pickavance Creek, Avalon Peninsula, Newfoundland.

1970

Number of Rows (Shapes)	=	5
Number of Columns (Colors)	=	5
Number of Levels (Species)	=	5
Number of Obs. . Per Cell	=	10

1971

Number of Rows (Shapes)	=	5
Number of Columns (Colors)	=	5
Number of Levels (Species)	=	5
Number of Obs. . Per Cell	=	6

Table of Variance

1970 - Black

1971 - *Italic*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value calculated	F value tabulated	Significance .05 = * .01 = **
A Color	4.0	14036.52	3509.13	4.02	2.39 3.36	* **
	<i>4.0</i>	<i>9695.03</i>	<i>2423.76</i>	<i>18.68</i>	<i>2.38</i> <i>3.34</i>	<i>*</i> <i>**</i>
B Shape	4.0	4839.86	1209.97	1.39	2.39 3.36	
	<i>4.0</i>	<i>810.57</i>	<i>202.64</i>	<i>1.56</i>	<i>2.38</i> <i>3.34</i>	
C: Species (Simuliidae)	4.0	61764.53	15441.13	17.70	2.39 3.36	* **
	<i>4.0</i>	<i>16150.46</i>	<i>4037.62</i>	<i>31.12</i>	<i>2.38</i> <i>3.34</i>	<i>*</i>

(Continued) . . .

Table of Variance (Continued)

1970 - Black

1971 - *Italia*

Source of variation	Degrees of freedom	Sum of squares	Mean square	F value calculated	F value tabulated	Significance .05 = * .01 = **
AB Inter- action	16.0	29995.93	1874.75	2.15	1.67 2.04	* **
	<i>16.0</i>	<i>2559.92</i>	<i>159.99</i>	<i>1.23</i>	<i>1.65</i> <i>2.01</i>	—
AC Inter- action	16.0	51912.53	3244.53	3.72	1.67 2.04	* **
	<i>16.0</i>	<i>21473.11</i>	<i>1342.07</i>	<i>10.35</i>	<i>1.65</i> <i>2.01</i>	* **
BC Inter- action	16.0	16968.58	1060.54	1.22	1.67 2.04	—
	<i>16.0</i>	<i>3612.80</i>	<i>225.80</i>	<i>1.74</i>	<i>1.65</i> <i>2.01</i>	*
ABC Inter- action	64.0	107172.24	1674.57	1.92	1.32 1.47	* **
	<i>64.0</i>	<i>6644.98</i>	<i>103.83</i>	<i>.80</i>	<i>1.30</i> <i>1.44</i>	—
Error	625.0	545389.67	872.62			
	<i>1125.0</i>	<i>145947.30</i>	<i>129.73</i>			
Total		832079.84				
		<i>206894.18</i>				

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REPUBLIKE SRBIJE



