BIONOMICS OF CULICOIDES (DIPTERA: CERATOPOGONIDAE) IN VIRGINIA

by

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FRONTISPIECE. Female Culicoides guttipennis preparing to feed.

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I. INTRODUCTION

"The most troublesome plague to both man and beast, especially in passing through the woods, was a kind of insect called by the Indians, Ponk, or Living Ashes, from their being so small that they are hardly visible, and their bite as painful as the burning of red-hot ashes." According to Jamnback (1965) the above quotation is from a report by Loskiel (1794, part III, p. 79) on the migration of the Christian Delawares from eastern to western Pennsylvania, and is perhaps appropriate to describe the severity of the bites of these vicious pests. The more common names used for these minute blood-sucking flies are "no-see-ums," "punkies" or "biting midges" in the mountainous or forested areas, and "sand flies" in coastal regions.

In many areas the ravages of these insects cause much greater concern than do mosquitoes (Culicidae). This is due to the severity of their bite and also due to the ability of the "no-see-ums" to enter dwellings through standard 16 x 18 mesh screening which normally excludes mosquitoes and other noxious insects.

In addition to allergenic dermatitis of horses and man caused by Culicoides bites, this group of insects are proven vectors of a number of other diseases in man or animals. Increased interest in Culicoides as vectors of disease has been expressed in recent times

but few possibilities have been investigated thoroughly. Basic knowledge on the host preferences and feeding habits of various species is essential prior to our determining their role as potential vectors. Laboratory colonization of various species would also be an invaluable aid in determining their role as potential vectors of certain diseases.

This group of minute biting flies has not received detailed study as have most blood-sucking diptera. This probably can be explained by the fact that the size of these pests not only makes them difficult to work with but also accounts for their being virtually unnoticed as facultative parasites of livestock. The need for study on the bionomics of Culicoides has been stressed by Kettle (1962) in a review article on the group, as well as Wirth (1964), and others.

In order to learn more about the bionomics of Virginia Culicoides the present study was undertaken from June 1964 to June 1966 with the following objectives:

(1) To determine the larval habitats of as many Virginia Culicoides as possible.

(2) To initiate colonization procedures on some of the readily obtainable species and to learn more about the life history of species that could be colonized.

(3) To ascertain an effective survey method for determining host preferences of various species of Culicoides and to collect preliminary data on host preferences of this group.

(4) To determine the periods of activity of some common species through light trappings.

II. LITERATURE REVIEW

A. Economic Importance

1. Annoyance.

Culicoides have annoyed man in all parts of the world for many ages and have depressed property values along some coastal resort areas due to their enormous numbers. According to Swanton (1922), Creek Indians escaped the ravages of these pests by placing smudge pots under their beds to ward off the hordes of blood seekers. Dove et al., (1932) believed that these biting insects were largely responsible for the lack of early development of the southern areas of the Atlantic Seaboard and cites a case in history where escaping Confederate soldiers who were forced to walk along the beaches of eastern Florida covered their bodies in sand and their faces with clothing to escape the annoyance of biting gnats and mosquitoes. These workers also reported the "giving up" of an escaped convict because he could not withstand the punishment of the sand fly bites. Hill (1947) indicated that the tourist business in the Highlands of Scotland suffered severely through the activities of certain species of Culicoides. The view has been expressed by Edwards et al., (1939) that a similar state of affairs existed in some parts of Great Britain. Dorsey (1947) gave an account of masses of C. peliliovensis

Tokunaga which produced severe lesions and thus not only reduced efficiency among troops, but which resulted in secondarily infected ulcerous wounds requiring hospitalization. Carpenter (1951), Woke (1954) and Blanton et al., (1955) have reported on the extreme annoyance to Panama Canal inhabitants. Two such reports are recorded from Virginia (Pratt 1907 and Murray 1957).

Severe dermal reactions occur in some individuals attacked by Culicoides. Notes on these reactions have been made by Jobling (1928), in detail by Hase (1933), as reported by Arean and Fox (1955), Carpenter (1951), Edmunds and Keener (1954), and Blanton et al., (1955).

A histopathologic examination of C. furens bites was made by Arean and Fox (1955) who referred to the lesions on newly arrived non-immune women and children at Fort Kobbe (Panama) as "Kobbe Leg." These workers described the severe local reaction of the skin of a patient to the bites of C. furens and gave the natural history of the lesion, histopathology and pathogenic mechanism involved.

2. Transmission of disease.

The greatest importance of Culicoides, however, is their ability to act as vectors of disease, especially filariasis. Culicoides were incriminated as possible vectors of tropical fevers as early as

1918 by Kinoshita. Stephens (1923), Purcell (1937), Wanson (1939) and Huttel et al., (1953) were in agreement with Kinoshita. In 1961 Fallis and Bennett compiled all reports on Ceratopogonidae as intermediate hosts for parasites and no new reports, before or since, were revealed through a literature survey. Their findings are presented in Table 1.

B. Bionomics.

1. Mating.

Many male nematocera aggregate to form mating swarms while in flight. Parker (1949) and Downes (1950, 1955), have studied swarms of several Culicoides in detail and have found that the swarms bear a definite relationship to a marker. Natural "swarm markers" given by Downes included pads of cow dung, patches of dark mud lying on a paler coloured road, or artificial markers consisting of black cloth 20-100 cm^2. Culicoides nubeculosus evidently does not form swarms, but rather mates on the host while the female is engorging (Pomerantzev 1932, according to Kettle 1962). Wirth (1964) speculated that the same procedure might occur in C. guttipennis.

Table 1. A review of the role of Ceratopogonidae in the transmission of disease agents. Compiled by Fallis and Bennett (1961)

Species	Parasite or disease	Distribution	Host	References
C. palpidipennis	Blue tongue virus	S. Africa*	sheep	DuToit (1944)
C. variipennis	Blue tongue virus	Texas*	sheep	Price & Hardy (1954)
C. nubeculosus	Onchocerca cervicalis	England*	horse	Baumann & Kment (1941)
		Europe		Enigk (1941), Molev (1955)
		Australia		Steward (1933)
C. pungens	O. gibsoni	Malaya*	cattle	Batelli (1954), Buckley (1938)
C. oxystoma	O. gibsoni	Australia	buffalo	Hopkins and Nicholas (1952),
C. shortii	O. gibsoni	India	Zebu	Howard (1904)
C. orientalis	O. gibsoni	Africa		
C. austeni	Dipetalonema perstans	Africa*	man	Duke (1954), (1956), Flock
C. grahami	D. perstans	S. Amer.		and Abonnenc (1949), Hopkins
C. inornatipennis	D. perstans	S. Amer.		and Nicholas (1952), Nicholas (1953), Nicholas and Kershaw (1954), (1955), Sharp (1928)
C. grahami	D. streptocerca	Africa*	man	Chardome and Peel (1949),
			chimpanzee	Henrard & Peel (1949)
			gorilla	Peel & Chardome (1946)
C. furens	Mansonella ozzardi	S. Amer.*	man	Buckley (1933), (1938)
				Flock and Abonnenc (1949)
				Mazzotti (1942)
Culicoides nr. piliferous	Haemoproteus nettionis	Canada*	duck	Fallis and Wood (1957)
C. sphagnumensis	H. canachites	Canada*	spruce grouse	Fallis and Bennett (1960)
Forcipomyia velox	Icosiella neglecta	France*	frog	Desportes (1942)

Table 1. -- (continued)

Species	Parasite or disease	Distribution	Host	References
		MORE UNCERTAIN STATUS		
C. erairai	Eczema	Japan*	man	Arnaud (1956)
C. robertsi	Allergic dermatitis	Australia*	horse	Riek (1954)
Culicoides sp.	Fowl pox	Asia	chicken	Bradley (1954)
C. pifanoi	Microfilaria	Venezuela*	?	Mirsa et al., (1952)
Leptoconops mediterraneus	Microfilaria	N. Africa	mule	Foley and Picot-LaForest (1923)
C. filariferus	1st stage larva (species unknown)	Mexico*	?	Hoffman (1939)
C. obsoletus	Cutaneous sores?	Japan*	man	Arnaud (1956)
C. arakawae	Leucocytozoon caulleryi	Japan*	chicken	Akiba (1960)
C. crepuscalaris	Haemoproteus sp.	Canada*	crow?	Fallis & Bennett (1960)
C. stilobezzioides	"	Canada*	crow?	" "
C. " "	"	Canada*	purple finch	" "
Culicoides sp.	Eastern equine encephalitis	USA*	?	Karstad et al., (1957)

* Distribution of some is limited and they are known host for parasites in certain countries only and are marked with an asterisk.

Mating is not essential for reproduction in all species as shown by Becker (1961) who obtained facultative parthenogenesis in C. circumscriptus.

2. Feeding and host preferences.

The ability of Culicoides to act as successful vectors of a disease depends greatly on its source of blood. Even though a few Culicoides are autogenous (Gluchova 1958, Williams 1961) most require a blood meal before egg maturation. Kettle (1962) believed that each species had a range of hosts on which it would feed, but generally preferred one. Also, certain species can be selective as to the area of feeding on the host. Kettle and Linley (1960a, 1960b, 1960c) and Kettle (1962) feel that this could be important in disease transmission when the parasites are restricted to a certain part of the body.

Female Culicoides have a great diversity of sources of the blood meal. Males are not haematophagus. In addition to birds and mammals, Culicoides have been collected on turtles, lizards and recently engorged mosquitoes (Edwards 1922, Myers 1935 and Downes 1958). Jamnback (1965) proposed a correlation between the abundance of sensory pits on antennal segments and host preference of Culicoides. He postulated that the antennae of ornithophilic species

have more olfactory pits than those that favor large mammals as the source of a blood meal. He had limited experimental evidence on which to base his hypothesis.

Birds as hosts of Culicoides have received increased attention in recent years. Reports on ornithophilic hosts were made by Painter (1927), Arnaud (1956) and Hicks (1959). Jellison and Philips (1933) collected Culicoides from crow and magpie nests and felt that Culicoides were important in transmitting filariasis in these birds. Judd (1954, 1957 and 1959) believed that Culicoides collected from the nest of catbirds had engorged from the birds. According to Wirth and Hubert (1960), similar collections of engorged C. ryckmani were made by Ryckman, Lee and Spencer from the nest of a house finch in a cactus at Indian Wells, California. Fallis and Wood (1957) observed biting midges feeding on white ducks in Canada and showed them to be intermediate host for Haemoproteus. Fallis and Bennett (1961) also collected "punkies" on spruce and ruffed grouse. In studies on ornithophilic blood-sucking Diptera in Ontario, Canada, Bennett (1960) collected six species of Culicoides, some in large numbers, on 1 or more of 13 hosts. This worker was able to determine the preferred ornithophilic host of several species.

The most commonly reported mammalian host of Culicoides has been man. Jamnback (1965) reported that 17 of the 37 species

found in New York had been reported feeding on man. Numerous other reports are listed for species not found in the Eastern United States. Messersmith (1961) compiled records of species feeding on mammals other than man until 1961. A summary of this survey is presented in Table 2. It should be kept in mind that these reports are of single observations in most cases and do not always represent a common or consistent relationship. In some cases the species was not known or determined.

In incriminating C. variipennis as a vector of bluetongue disease of sheep, Jones (1961) obtained conclusive evidence that this species of biting midge feeds on sheep in nature. Jones used a portable animal-baited trap in his studies. In a later study, Roberts (1965) collected the following species of Culicoides in a steer-baited trap: biguttatus, crepuscularis, travisi and variipennis.

3. Ovarian development and oviposition.

Amosova (1959) and Glukhova (1958) found that one full blood meal is sufficient for maturation of the ovaries of Culicoides. According to Kettle (1962) Linley (unpublished data), has shown the ovarian cycle in Culicoides to be similar to that of mosquitoes and ovarian development proceeds only up to a certain stage prior to a blood meal unless the species is autogenous. Hill (1947) found that

Table 2. Review of hosts of Culicoides (From Messersmith, 1961)

Hosts	Country	Reference
Horses	England S. Africa, Panama U. S., Australia England	Steward (1933), DeToit (1944), Carpenter (1951) Pickard and Snow (1955), Lee (1956), Campbell and Pelham-Clinton (1960)
Cattle	Malaya, England Australia, U. S. England	Smith and Swaninath (1932) Buckley (1938), Hill (1947) Lee (1956) Jones (1959) Campbell and Pelham-Clinton (1960)
Sheep	S. Africa, U. S. Australia	DuToit (1944), Price and Hardy (1954), Jones (1959) Lee (1956)
Goats and Pigs	England	Campbell and Pelham-Clinton (1960)
Monkeys		Causey (1938)
Rabbits	Australia, U.S.	Lee (1956), Jones (1959)
Mice	U. S.	Jones (1959)

flies would take blood no later than 2 days prior to egg-laying and that some flies took seven blood meals prior to oviposition. The taking of a second blood meal within 1 hour after the first by Culicoides has been recorded. Maturation of eggs is rapid after a blood meal and oviposition occurs within a few days (Myers 1935, Downes 1950, Lewis 1958, Jones 1960 and Jamnback 1961). Downes (1950) found that C. nubeculosus oviposited 4-5 days after a blood meal and immediately took a second blood meal, resulting in another batch of eggs. This worker obtained five egg batches from one female. Gulchova (1958), as reported by Kettle (1962), found that the number of eggs varied with the size and quality of the blood meal. Even though Parker (1949), Jobling (1953) and Lewis (1958) stated that the usual number of eggs per batch is less than 100, Jones (1965) in studies with C. variipennis has found that this number can be greatly exceeded. Jones collected a maximum of 243 per female with C. variipennis.

Most Culicoides exercise considerable care in selection of an oviposition site and certain types of larval habitats can be associated with certain species (i.e. C. guttipennis with wet tree and stump holes). In absence of proper conditions, females will refuse to oviposit but can be forced to do so by decapitation (Becker 1956). Downes (1950) showed that singly isolated females failed to oviposit

whereas others confined together oviposited overnight. Kettle (1962) believed that this density dependent factor could account for high local concentration of larvae in some areas.

4. Sampling procedures for immature and adult Culicoides.

Surveys for immature Culicoides can be conducted using a variety of different techniques, depending on the type of habitat. Dove et al., (1932) used adult recovery cages which were placed over suspected breeding areas of salt marsh sandflies. Other workers utilizing a similar device include Sailer et al., (1956) and Williams (1956). Buckley (1938) located the breeding places of Culicoides in Kaula Lumpur, Malaya, by taking samples of mud and placing them in gause-covered cylinders. Breeding in muck soils has been studied by Williams (1951) and Dove et al., (1932), who placed quart samples of soil in a small dish and then submerged them in a larger bowl of water. Larvae left the soil sample overnight and could be observed swimming in the surrounding water. Other methods include the magnesium sulfate method and the sand migration method employed by Bidlingmayer (1957). In isolating larvae of salt water species from sand, Jamnback and Wall (1958) collected 1/3 pint sand samples and added an equal quantity of water. This was mixed by agitating the water briefly. The water was then poured off as the sand settled. The larvae, if present, could be observed swimming in the supernatant.

In adult surveys the most common device used has been the New Jersey type light trap. James (1943) was probably the first to employ this method. Since that time world-wide use has occurred: (Lee (1956) - Australia; Williams (1956); Downes (1958)-Canada; Arnaud (1956) - Japan; Campbell and Pelham-Clinton (1960) - England; and numerous others, including extensive use in the United States). Specimens are generally collected directly into 70% ethanol.

Collecting of adults from man by aspiration has been used widely and is effective for survey of a number of species (Buckley (1933), Pickard and Snow (1955), Williams (1955), Murray (1957), Snow et al., (1957), Wirth and Hubert (1962) and many others). Even though this method is widely used it has its disadvantages in that some species have not been collected on man (i.e. C. arboricola and C. stellifer (Jamnback 1965).

Animal-baited traps hold promise as being a fairly effective tool for adult survey and have been used in a number of instances in recent years. Fallis and Wood (1957), Bennett (1960) and Fallis and Bennett (1960) have used this type of trap very successfully. Jones (1961) and Roberts (1965) have employed animal-baited traps and found them effective in surveying for ectoparasites of large animals.

Nelson (1965) found that C. variipennis occidentalis and C. variipennis sonorensis were attracted to carbon dioxide baited traps

and could be collected in large numbers. Possibly other species express this CO_2 tropism which is significant in the detection of vertebrate hosts. A modified New Jersey light trap baited with CO_2 has been used recently by Snoddy and Hays (1966) to trap other biting flies (Simuliidae). These workers made no mention of Culicoides or other insects collected.

C. Larval Habitats.

Our knowledge of Culicoides breeding sites has increased greatly in recent years, but as Williams (1964) pointed out, the larval habitats of the majority of the world species of Culicoides are unknown. He also mentioned that for those that are known, the habitats are varied and frequently only a single habitat is known for a given species. Culicoides are known to utilize a wide variety of habitats for oviposition, and all habitats have at least one thing in common -- all are moist or wet. The literature available on breeding sites of Culicoides pertinent to this study is presented in Table 3. Other habitats include slime-covered tree bark (Thomsen 1937), decaying fruit and stems of plants, coconut shells, debris in pitcher plants, crab holes (Wirth and Blanton 1959), Cacti (Ryckman 1960), rotting pods of cocoa, Bromeliads, decaying flowers of Balisier, rotting calabash, banana stumps and others (Williams 1964).

Table 3. A review of the breeding sites of Culicoides pertient to this survey

Species	Habitats	References
arboricola Root and Hoffman	wet tree-holes, wet wood debris, water and moist woody debris	Jones (1961), Root and Hoffman (1937), Wirth and Bottimer (1956), Snow et al. (1957).
biguttatus (Coquillett)	decaying leaf muck reservoir margins and leafy pools, sulfate waste basin, flooded wooded bottom, rain pool on mud flat, vegetated stream margin.	Williams (1955), Snow et al. (1957), Murray (1957), Wirth (1951).
crepuscularis Malloch	Carex peat and organic mud, mud, sand at pond margins, water tank overflows, septic tank effluent.	Williams (1955), Wirth and Bottimer (1956), Jones (1961), Snow et al. (1957).
footei Wirth and Jones	oak and maple tree-holes, tree-hole debris	Wirth and Jones (1956).
furens (Poey)	salt marshes, bays, drainage ditches, wetted areas subject to flooding.	Wall and Doane (1960), Bidlingmayer (1957), Woke (1954), Williams (1964), Goulding et al. (1953).
guttipennis (Coquillett)	tree holes, crotches, stumps.	Jones (1961), Murray (1957), Root and Hoffman (1937), Messersmith (1961), and others.

Table 3. -- (continued)

Species	Habitats	References
haematopotus Malloch	spring seepage areas, margins of streams, rivers, and lakes, alkaline streams, over flow areas at watering trough, mud flat, woody debris, muddy sandbar, sandy stream margin.	Jones (1961), Murray (1957), Snow et al., (1957), Jamnback (1965), Wirth (1951).
hinmani Khalaf	moist debris in tree-holes.	Snow, et al. (1957), Wirth and Bottimer (1956), Wirth and Jones (1956).
hollensis Melanders and Brues	salt marshes, drainage ditches, bays, all with vegetative cover.	Jamnback (1965), Jamnback et al. (1958), Wall and Doane (1965).
melleus Coquillett	intertidal sand.	Goulding et al. (1953), Wall and Doane (1960), Jamnback et al. (1958).
nanus Root and Hoffman	tree-holes	Snow et al. (1957), Jones (1961), Wirth and Bottimer (1956), Root and Hoffman (1937), Messersmith (1961).
obsoletus Meigen	clean straw beside a chicken house, spruce needles, twigs, and wood chips.	Jamnback and Wirth (1963).
piliferus Root and Hoffman	osmunda fernbog, stream edge, mud with grass, soft mud, sand and silt mixture.	Wirth and Hubert (1962). Jamnback (1965).
sanguisuga Coquillett	moist leaves on well drained sites, straw near chicken house, spruce needles, twigs and wood chips.	Jamnback and Wirth (1963).

Table 3. -- (continued)

Species	Habitats	References
snowi Wirth and Jones	debris in tree holes.	Wirth and Jones (1956).
spinosus Root and Hoffman	grassed boggy streams, mud bars, pond margins, flooded wooded bottom, mud flat.	Jones (1961), Wirth and Bottimer (1956), Snow, et al. (1957).
stellifer Coquillett	mud and wet soil with decaying leaves and roots, grassy margin of lake, mud flat, decaying leaves at pond margins.	Williams (1955), Snow et al. (1957), Murray (1957), Wirth and Bottimer (1956), Jones (1961).
travisi Vargas	wet grass and mud from marsh of meadow, cattail swamp, leaves and detrites from stream margin, mud bars leafy pools in wooded area.	Jamnback (1965), Jones (1961), Snow et al. (1957).
variipennis variipennis Wirth and Jones	manure contaminated areas around livestock watering tanks.	Wirth and Jones (1957).
venustus Hoffman	stream margins, mud or mud with grass roots where depressed by cattle hoofs. Sphagnum moss.	Jamnback (1965), Jones (1961), Snow et al. (1957).
villosipennis Root and Hoffman	wet tree-holes.	Jamnback (1965), Jones (1961), Root and Hoffman (1937), Snow et al. (1957).

D. Laboratory Rearing and Colonization Procedures.

Even though numerous workers have attempted to rear and colonize members of the Culicoides group, only two reports have appeared on successful colonization (Downes 1950 and Jones 1957, 1960). Much of the limited biological data that we have on the group have come from laboratory rearing of field collected material. Gravid females have been the most common starting place and a number of workers have collected data on parts of a single life cycle using field collected material. Patel (1921) reported that eggs were recovered from field collected C. oxystoma when engorged females were supplied with moist filter-paper after a maturation period of 50-60 hours. Even though larvae were obtained, future attempts at propagation evidently failed. Dove, Hall and Hull (1932) reared field collected larvae of C. canithorax and C. dovei to adulthood by feeding them decaying grass-roots and humus and supplying a small amount of brackish water two times a week. They believed temperatures above 70°F were detrimental to the larvae.

Observations on the development of C. nubeculosus from egg to adult were made by Steward (1933). This worker obtained oviposition on horse manure and damp soil, and reared the larvae to adulthood on tap-water containing bacterial scums resulting from the horse manure. Atchley and Hull (1936) obtained oviposition by wild

caught specimens of C. canithorax, C. dovei and C. melleus on moist filter-paper or moist marsh soil in a petri dish when adults were housed in glass lamp-chimneys. Eggs from these species hatched 4-5 days after oviposition, but resultant larvae died within several days, even when kept in marsh soil. According to Hill (1947), Jobling (1953) reared C. vexans on heavy loam soil but was unsuccessful in maintaining the culture for more than one generation.

The first species of Culicoides to be colonized was C. nubeculosus and was first referred to by Downes (1950) who stated ". . . C. nubeculosus is being maintained in the laboratory . . . ." Further information was not given. Roberts (1950) also referred to the same colony but did not elaborate on colonization procedures. Megahed (1956) gave culture methods for this species and gave credit to J. A. Downes of the University of Glasgow for its establishment in 1947. Megahed indicated that the life cycle was completed in from 3 to 8.5 weeks. Larvae were reared on a simulated natural diet and required dried, powdered soil from the original source, together with yeast and powdered charcoal. C. nubeculosus was maintained in the laboratory from 1947 to mid-1953 according to Megahed (1956), at which time it began to show symptoms of deterioration. The gravid females refused to lay eggs and the size of egg-batches and the percentage that hatched became smaller.

Apparently the first highly successful colonization attempt was that of C. variipennis sonorensis by Jones (1960). Jones had also previously maintained this species in the laboratory for 12 consecutive generations but large numbers were never produced. In his 1960 paper, Jones gave a routine procedure for propagation of 1000 adults per day and stated that viable-egg production had exceeded the number required to insure colony preservation. He listed colonization equipment and the procedures for handling adults. Jones (1965) gave the life cycle of C. variipennis as about 24 days -- egg 2, larva 15, pupa 3, period prior to blood ingestion 1, and preoviposition period 3. Adults and larvae were held in a room maintained at 75°-80°F and a relative humidity of 40% to 50% (Jones 1960). Jones (1965) has developed a simulated natural larval diet and used a small amount of suitable soil to provide microorganisms, vermiculite as a substrate and cow manure as a source of detritus and nourishment for the microorganisms. Water was also added to the mixture.

III. METHODS AND MATERIALS

A. Procedures Used in Larval Surveys.

Several methods were used in determining the breeding sites of Culicoides larvae. Emergence traps similar to those described by Dove et al., (1932) were used to a very limited degree in these studies. They were not suited for certain habitats (i.e. tree holes) and were often unsatisfactory in other types of habitats. Those few emergence traps used were constructed in accordance with the instructions of Dove et al., (1932) with the exception of the top panel. The black muslin and screen top was replaced with masonite. The recovery cages were 24 inches square and 16 inches high. A galvanized metal strip serving as a cutting edge was tacked around the bottom of the cages and could be pushed into the soil to hold the cage firmly in place.

The most generally used technique, and by far the most satisfactory, was a modification of the technique used by Buckley (1938) and is described below. During the summers of 1964 and 1965 many likely breeding sites of Culicoides were visited throughout most of Virginia. Several 2-quart samples of mud, leaf debris or similar solid materials collected from prospective breeding sites were placed in 1-gal food containers coated inside with paraffin. When

probable sites, such as tree-holes and stump-holes were sampled, attempts were made to remove the entire contents of the tree or stump-hole. Pond edges were sampled by scooping up mud and soil for a few inches along both sides of the water line. These samples were retained in gallon food cartons in the same manner as mentioned previously for solid materials. In the laboratory the solid tops of the food cartons were replaced with screen tops of 40 mesh saran screening. A small stoppered hole in the center of the screened top permitted easy removal of adults upon emergence by aspiration. All samples were held at 75°F ± 3° in a well lighted room. Distilled water was used when needed to moisten solid samples to prevent drying out before adult emergence was completed. Adults were removed at least once daily and killed in 70% ethanol.

B. Laboratory Colonization Procedures for Culicoides guttipennis.

The need for laboratory colonies of Culicoides in order that we may learn more about the bionomics, control and potential as vectors has been stressed by Jones (1960) and Kettle (1962). Jones stated "The colonization of other species will not only increase our knowledge of Culicoides biology, but will assist in determining the role of these flies as vectors of animal diseases." Kettle (1962), in his review article on Culicoides, said ". . . bionomics and

control of Culicoides and Leptoconops is still in its infancy. There is urgent need for laboratory studies on the behavior and physiology of different species. This, in turn, awaits the establishment of laboratory colonies."

Numerous C. guttipennis larvae were collected during April, 1965, from hollow trees and stumps from the vicinity of Blacksburg, Virginia, and brought into the insect rearing room at Virginia Polytechnic Institute. Larvae were held at room temperature in the original water and debris in which they were collected from their natural habitat. As adults emerged, they were removed from the emergence container and transferred to constant temperature cabinets. Although all procedures, and thus production, were on a very small scale at the beginning, they have developed into a relatively large, and productive process.

1. Adult maintenance.

Adults were maintained in constant temperature cabinets at 80° F ± 2° and 85% relative humidity ± 10% in semi-darkness (less than 1 ft-c) at all times except while being attended.

Adult holding cages (Fig. 1) were constructed of 1/2 gal cardboard food containers, fine-mesh nylon stocking, transparent polyethylene and cork stoppers. A number of holes were cut in the

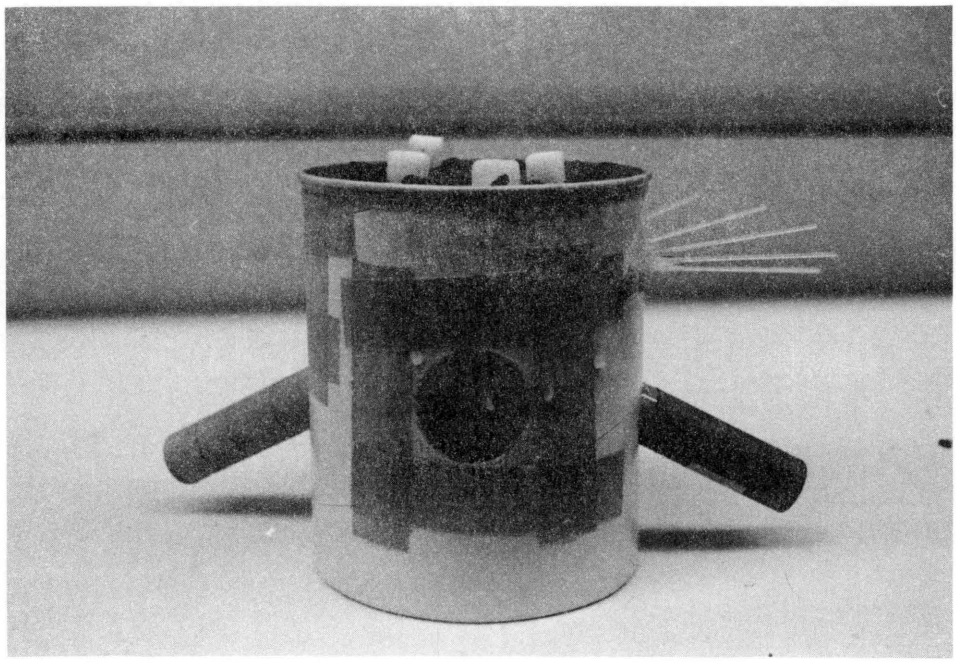

Fig. 1. Adult holding cage for C. guttipennis constructed from a ½ gal food container.

cardboard container. A 2 inch front view window was cut about 2 inches from the top of the cage and was covered with transparent polyethylene taped in place. A 1/2 inch polyethylene covered hole cut near the top rim on one side provided an entrance for a beam of light used to attract the females to the top of the cage during blood feeding procedures. Two other 3/4 inch holes located about 2 inches from the bottom (at any position) were entrances for egg collecting vials and were stoppered during blood feedings. One other hole stoppered with a small cork provided an entrance for adding newly emerged adults to the parent cage. From 10-15 minute holes were bored in the side of the cage and allowed for the entrance of capillary tubes containing liquid diets. These holes were covered by a small piece of tape when capillary tubes were removed during blood feeding. The top of the carton was covered with tightly stretched, fine-mesh nylon stocking which was held firmly with rubber bands and glue.

Adults were afforded a number of different diets. Rabbit blood was offered the adults daily from the time of emergence. The feeding procedure was similar to that described by Jones (1960). It differed, however, in that in our laboratory the females fed through the stockinged cage top on a closely shaven belly of a rabbit (Fig. 2) and there was no loss of adults through anesthesia or escape. Split raisins and sugar cubes were placed on the stockinged top of the adult

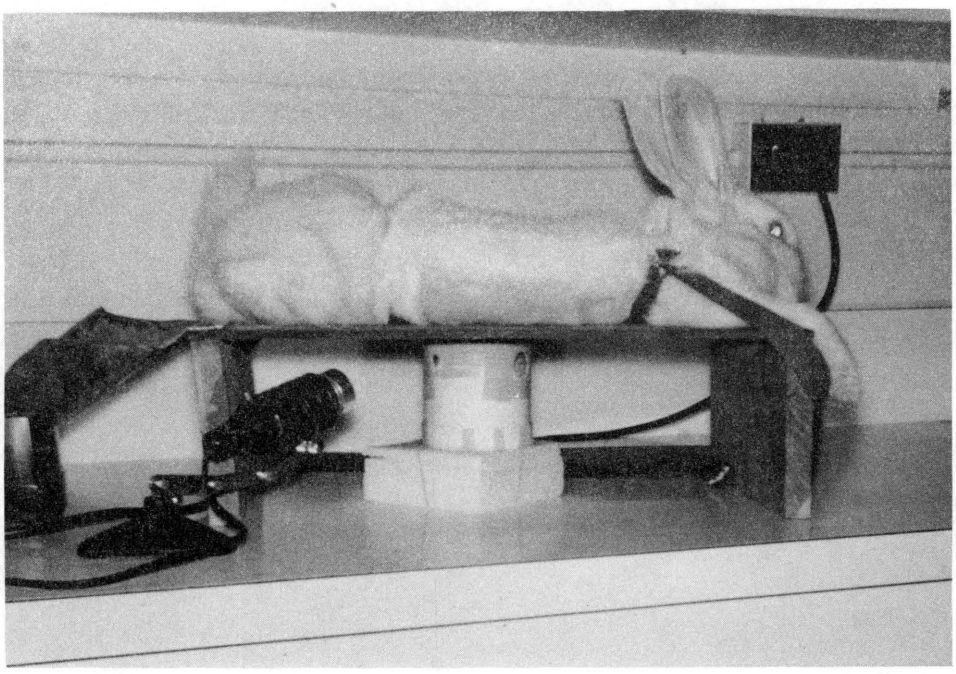

Fig. 2. Materials used in the blood-feeding of adult C. guttipennis.

holding cage between blood meals. A fourth type of diet was the adult house fly diet described by LaBrecque and Gouck (1963). This was mixed with an equal weight of water and was administered in capillary tubes through the side of the cage. A 10% honey water solution was fed likewise.

2. Egg collecting.

When the first adults in a cage reached 4 days of age, two dram shell vials containing filter paper and "artificial stump water" described below were inserted through the side of the cage. The filter paper was rolled to fit the inner surface of the vial and the vial was filled about 1/2 full with water which had been allowed to stand on decaying leaves and organic matter for a week or more. The water had attained a characteristic brown color and had a sour smell. It resembled water frequently found in hollow trees and stumps in nature.

The egg vials were removed daily, or every other day depending on the production of the females, and placed upright in a 5x5x8 inch plastic box containing a small amount of distilled water. They remained in this humid condition until they began to hatch at which time the filter paper and water contents of the vial were transferred to larval rearing chambers.

3. Larval rearing chambers.

Larval rearing was conducted at 87° F ± 3° in complete darkness except for the light that diffused through the emergence cups. Four gallon aquaria were used as larval rearing chambers. The sides were covered on the outside with black polyethylene or painted black to exclude all light. The top was likewise covered with black polyethylene which was taped in place. Two holes were cut in the top of the chambers. One about 1/8 inch in diameter was the entrance for an air hose used to break up surface scums and the other was the size of a pint cardboard carton. A cardboard carton was modified so that the bottom of the carton and top panel of the lid were replaced with 40 mesh saran screening (Source: National Filter Media Corp., 1717 Pixwell Ave., New Haven 14, Conn.). A slit 1/8 inch wide was cut across the bottom screen. The emerging adults were attracted to the lighter screened area where some light diffusion occurred and crawled through the slit into the container. The cups were then removed and transferred to a refrigerator for cooling of adults for a short time, after which the adults were easily transferred to the adult holding cages.

4. Larval media.

The larval media consisted of decaying leaf-mold collected in hardwood forests in several areas near Blacksburg, Virginia. The most satisfactory debris was that at least 1 year old. Bottoms and gullies where deep leaf accumulations existed afforded the best collecting sites of leaf mold. The newly-fallen leaves were raked back and the decaying humus collected for use. Work on artificial diets has been initiated, but a completely satisfactory diet has not yet been developed.

From 1 to 1-1/2 gal of leaf mold was placed in each aquarium and held at the bottom with a frame covered with screen (Fig. 3). Two gallons of distilled water were then added and the aquarium was placed at 87° F ± 3° for 1-3 days before newly-hatched larvae were introduced. The evaporation rate was low and generally no additional water was added. The aquaria contents could be used for a second generation of larvae, but this practice often resulted in unsatisfactory conditions for the second batch.

C. Host Preference Studies.

The works of Fallis and Wood (1957), Fallis (1958), Bennett and Fallis (1960) and Fallis and Bennett (1960) on host preferences of ornithophilic species of Culicoides have been the most extensive

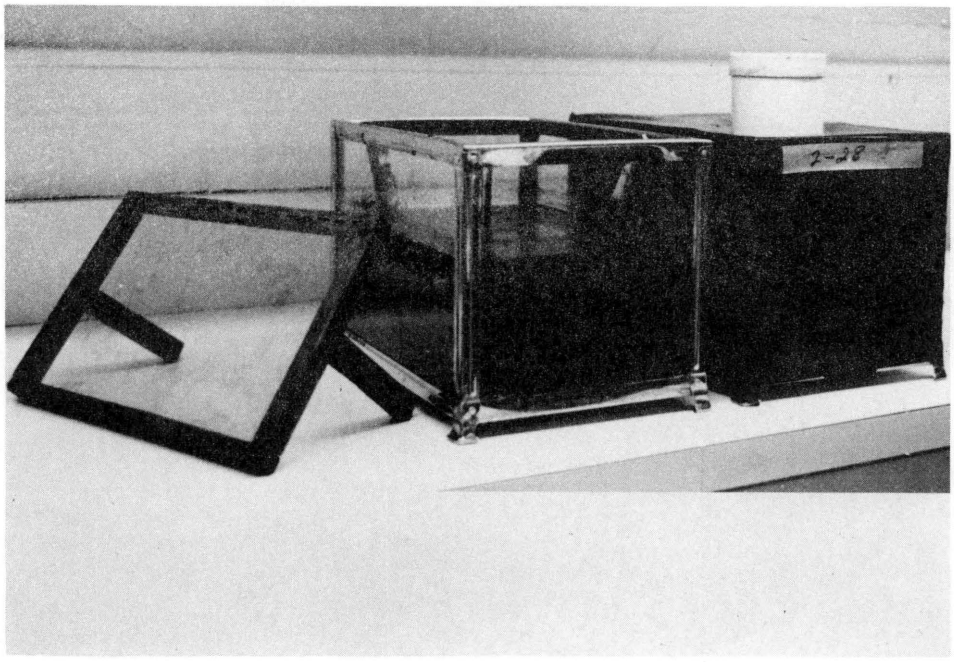

Fig. 3. Materials used in the preparation of larval rearing chambers for C. guttipennis.

studies on host preferences for this group of blood-sucking flies. These workers used only a few hosts and all were fowl. Because of the almost complete lack of data on host preferences, the present study utilizing as many as 14 different hosts, was undertaken. Failures in disease transmission studies of avian infectious synovitis by Turner et al., (1963) and of eastern viral encephalitis and vesicular stomatitis by Lee (1962) can possibly be attributed to the utilization of improper species as potential vectors. Lee (1962) stated that possible guides as to species to consider should result from further knowledge of host preferences for species of this group.

1. Hosts and sites used in this study.

Due to inadequate knowledge as to the host specificity of certain Culicoides it was felt that in preliminary investigations as many hosts as practical should be used in as many different types of habitats as feasible. Bennett (1960) has shown that some ornithophilic species have specific preferences (i. e. upland birds, ducks, etc.). Due to the large number of hosts used, the limited manpower, and the time involved in a single trapping, only one replicate was run at each site. Fourteen hosts, including domestic rabbit (Oryctolagus), eastern cotton-tail rabbit (Sylivilagus), guinea pig (Cavia), opposum (Didelphis), domestic white rats (Rattus), chicken (Gallus), domestic

turkey (Meleagis), mallard duck (Anas), bobwhite quail (Colinus), mourning dove (Zenaidura), common box turtle (Terrapene), southern painted turtle (Chrysemys), bull frog (Rana) and man were used in seven different habitats. Habitats included a wooded area near a small wildlife watering pond in Broad Run Game Reserve near New Castle, Virginia. This site was utilized most extensively due to its accessability and productivity for a number of different species of Culicoides. Two sites were selected near Newport, Virginia. One of these was on the bank of a fresh water pond and was surrounded by dense forest. The other site at Newport was on Craigs Creek near Virginia Route 460. Other sites included an upland wooded area near Blacksburg, a peat bog area at Cranberry Glades, West Virginia, a wooded mountainous area near several stream tributaries at Vesuvius, Virginia, and a salt marsh at Virginia Beach, Virginia.

2. Traps and trapping procedure.

Two types of bait-traps used in these studies have been described in the literature. Bellamy and Reeves (1952) have described a lard can type bait trap which was modified for our use in Culicoides studies. Basically this type of trap is a metal can with screened funnels leading inward at both ends. Forty-mesh saran screening was used in the construction of the funnels for 10 gal cans.

The second type of trap has been described by Bennett (1960) and has three basic parts. The first is a restraining cage for the animal and is constructed of large mesh fish net (1/3-1 inch mesh) or chicken wire. The restraining cage is placed on a white plywood base and covered with a collecting cage after exposure of the animals. The collecting cage consisted of a frame covered with saran screening (40 mesh) or transparent plastic on five sides, leaving the bottom open. Plastic or rubber sponge glued to the bottom frame of the cage formed a tight seal and prevented escape of the flies. Flies were removed by aspiration through an opening in one end of the cage. The opening was covered when not in use. Even though most cages used were covered with transparent plastic, it is now felt that the fine mesh screening would probably be superior. Infrequently there were moisture condensation problems with the plastic covered collecting cages. Since the lard can type traps previously described (Bellamy and Reeves 1952), and the three other traps constructed and tried in these tests were entirely unsatisfactory, all trappings after initial tests were conducted using "Bennett" traps (Bennett 1960).

The trapping procedure with the Bennett traps involved placing the host animals in restraining cages on the plywood bases, at random, in a habitat fairly uniform in vegetative cover, exposure to light, etc. Animals were placed about 15 ft apart. An acclimation

period of 15 min was allowed after the last animal was exposed before the experiment was begun. Workers left the immediate area after the last animal was placed on a white base. It was hoped that this acclimation period would minimize the effect of man having been in the area and acting as an attractant. After the acclimation period, all hosts were exposed for 15 min (Fig. 4) and then quickly covered with the collecting cages. Culicoides spp. visiting man during this 15 min period were collected from the bare arms by aspiration. Culicoides visiting other hosts were given a 15 min period after placement of the collecting cages to complete feeding before insect collections from the cages were begun. Generally a flashlight was required in the collecting of the flies from cages since activity and trapping began just prior to darkness. The light also aided in the collection of flies due to the phototrophic nature of almost all species. Culicoides had to be aspirated from the cages which was both laborious and time consuming at night. All specimens were stored in 70% ethanol.

When a second or third exposure of animals was made on the same night, all animals were left covered with collecting cages until the last "punkies" were removed from the previous collecting period and then all hosts were exposed simultaneously. Again, an acclimation period of 15 min was allowed prior to starting the next test.

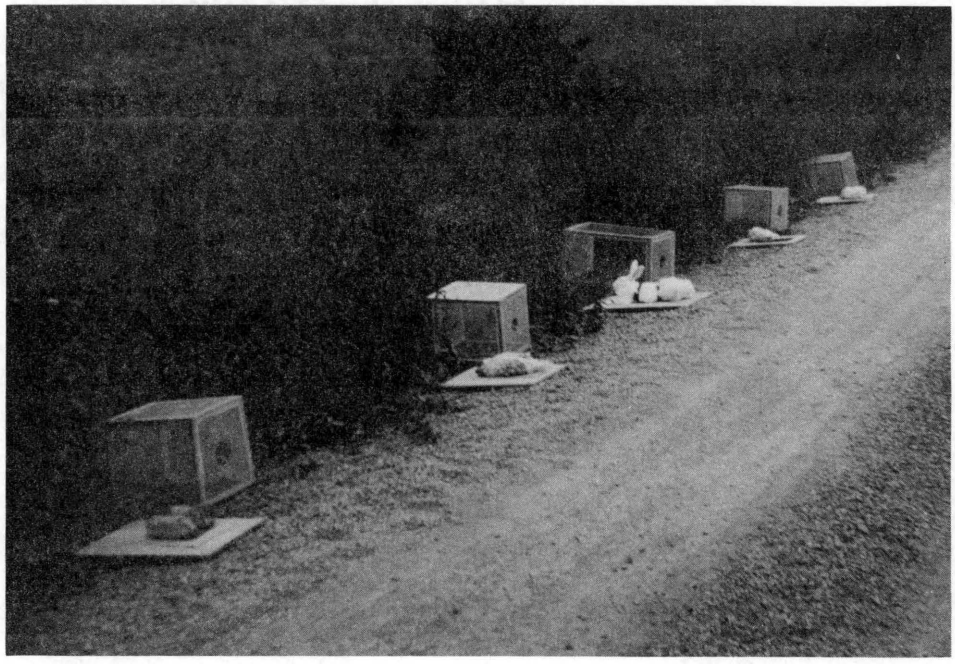

Fig. 4. "Bennett type" animal baited traps used in host preference studies.

D. Adult Activity Studies.

Using light traps, Williams (1955b) collected a small amount of data on the daily activity of nine species of Culicoides. He used four traps operating in 3-hour cycles from 6 PM to 6 AM. The present study was set up similarly since it was felt that activity data would be quite useful in relation to host preference or feeding habits studies.

Three standard New Jersey light traps were used in these studies. With the assistance of Mr. U. F. Earp of the Agricultural Engineering laboratory, Virginia Polytechnic Institute, a time switch was installed on each trap and received alternating current from a Powercon® vibrator inverter (Cornell-Dubilier Electronics, Fuquay Springs, N. C.) which operated on direct current from a 12-volt auto battery.

An upland wooded area near the College of Agriculture, Virginia Polytechnic Institute, beef barns was selected for this study. A small stream was situated several hundred feet away and provided breeding grounds for a few species. All three traps were located in very close proximity and set to operate during one of three time periods. One trap began operation each night at 9 PM (DST) and operated until 12 PM. The other two were also set for 3-hour periods and started operation at 12 PM and 3 AM respectively. Specimens

were collected in 70% ethanol. Eleven collections were made from June 8 to August 30 during the summer of 1965.

E. Preparation of Specimens for Identification.

Due to the minuteness of most species of Culicoides they are difficult to handle and identify unless mounted on microscope slides for study. Wirth (1964) prepared instructions for preparing slides of Ceratopogonidae and Chironomidae in March 1959. This procedure was also given and recommended by Wirth and Blanton (1959). This procedure involved clearing the flies in phenol, dissection of the insect into head, thorax, one wing and abdomen in the female, and the additional removal of genitalia in males. The insects were dissected on the slide and mounted in a 50-50 phenol-balsam mixture. Trained technicians were necessary for this procedure.

IV. RESULTS

A. Larval Surveys.

According to Messersmith (1965) there are 32 species of Culicoides known in Virginia. As was shown by the present survey, they occupy a great diversity of habitats. One or more breeding sites for 22 species were located and recorded for Virginia in this study. Breeding sites were divided into broad, generalized headings as follows: Tree and stump-holes (wet vegetative habitats); saline habitats; moist terrestrial habitats, (a) mostly alkaline, dung polluted, and generally exposed to direct sunlight; and (b) generally acidic, soil and plant habitats other than tree holes. Nine species of Culicoides were found breeding in first type of habitat, four in the second, six in the third and eight in the last type.

1. Description of breeding sites and Culicoid fauna present.

Tree and stump-holes. - In this environment there exists two distinct sets of conditions which will influence the species of Culicoides present. Wet tree or stump-holes are defined as cavities which retain water even in periods of dry weather. Stumps of white oak, Quercus alba L. were common and supported large numbers of one or two species. Dry tree-holes are generally more protected from rain and evaporation and excessive rainfall drains off. The

dry tree-holes most productive in this study had a small opening leading into a large hollow containing moist decaying organic matter.

C. arboricola. - In this survey C. arboricola was found in both wet and dry tree-holes. At least two records were made of its presence in wet white oak stumps where it appeared in small numbers with C. guttipennis. These stumps were located in Montgomery and Rockingham Counties and contained decaying leaves, other organic material and some soil which was covered with dark-brown water. Larger numbers of C. arboricola larvae were found in a drier habitat in a hollow yellow buckeye tree (Aesculus octandra March) near Newport, Virginia (Giles Co.) (Fig. 5). A small, slit-like cavity facing north opened into a 3-4 gal hollow which was filled with peat-like organic matter. The non-compacted substrate contained only enough water to make it moist. A number of earthworms, slugs, and dipterous larvae were present which probably kept the substrate well aerated. Three other species: C. hinmani, C. stellifer and C. footei were found breeding in conjunction with C. arboricola in this case.

C. footei. - This species was taken in rather large numbers on May 28, 1965, from the same yellow buckeye tree cavity mentioned under the heading C. arboricola. According to Wirth (1965)

Fig. 5. Hollow yellow buckeye tree which supported larval populations of Culicoides arboricola, C. footei, C. hinmani and C. stellifer.

this was the first report of this species having been taken since its original description in 1956. Future collections from the site yielded mostly C. guttipennis since the habitat changed from a dry to wet habitat upon removal of the organic debris.

C. guttipennis. - This was found to be the most abundant tree and stump-hole breeder in Virginia. This can possibly be explained by its ability to survive under a wide range of environmental conditions. Temperature, pH, and dissolved O_2 readings indicated the ability of this species to survive under the following conditions: temperature range 2.7 to 30.4°C; pH 5.0 to 8.2; and dissolved O_2 7.0 to 10 ppm. C. guttipennis was found abundantly in both wet and dry tree and stump-holes and comprised over 95% of all culicoid fauna collected from wet tree and stump holes. Mature larvae were found and removed from a white oak stump in Blacksburg on April 27, May 18, June 5 and 29, August 8, September 1, and September 20. Larvae were not present in this stump in July. Otherwise there was almost continuous breeding of this species during the summer months of 1965 in Southwestern Virginia. Breeding sites in trees and stumps were so extensive and varied that they will not be described further.

C. hinmani. - This species apparently prefers dry tree-holes for oviposition and is relatively scarce as indicated by light trap surveys made during 1959-1960 (Messersmith 1961, 1965). However, collections on live animal hosts used during 1965 in another study have shown this species to be somewhat more prevalent than previously indicated by the above author. Only one breeding site, the same as described for C. footei, was found for this species during the summers of 1964 and 1965. About 100 male and female specimens were collected from the buckeye tree.

C. nanus. - Small numbers of this species have been collected in Rockingham County from two white oak stumps containing water and an accumulation of organic debris. Messersmith (1961) also reared a few specimens from stump water collections which might indicate that this species prefers a wet larval habitat somewhat similar to C. guttipennis. C. guttipennis larvae were abundant in both of the C. nanus collections.

C. stellifer. - Specimens were collected from the same hollow buckeye tree habitat described for C. footei and C. hinmani. About 25 specimens were collected from the site.

C. sanguisuga. - A single specimen of this species was collected from a wet white oak stump in Rockingham County near Ottobine, Virginia, on April 24, 1964. As Table 3 indicates, this was an unusual habitat and probably accounted for the presence of only a single specimen.

C. snowi. - This tree-hole breeder was encountered only once in this survey. A single specimen emerged from water and debris taken from a white oak stump in Rockingham County on April 24, 1964. C. guttipennis was found in large numbers in the same stump.

C. villosipennis. - This species is evidently a wet stump and tree-hole breeder. Second to C. guttipennis, it was the most abundant species recovered from wet stumps and tree-holes. It was reared in moderate numbers from stump water taken near Wytheville (Wythe Co.), Ottobine (Rockingham Co.) and New Castle, Virginia (Craig Co.). All stumps were white oak and characterized by having organic debris covered by at least several inches of water. In all instances C. guttipennis was also present.

Saline Habitats

In addition to the coastal region of Virginia which supports at least three species of Culicoides, the brine pools near Saltville, Virginia, provided an inland saline habitat in which a fourth species was found.

C. variipennis australis. - The presence of this species at Saltville was reported by Snow et al., (1957) and Messersmith (1961). In accordance with the findings of these workers, the areas of greatest larval concentration were algal mats (Lyngbya and Anacystis) in moist depressions and drainage ditches. During June 1964, larvae were so numerous in these algal mats that the entire surface of the algae glistened in the sun due to larval movement. Thousands emerged from half-gallon samples of mud and algae. An Olin Matheson Company foreman of the area stated that frequently the brine of the saline pools in the Culicoides breeding area contained concentrations of salts above 250 parts/mille. Most areas were exposed to direct sunlight. This subspecies was not found along the coast.

C. hollensis. - Even though extensive surveys of the Virginia Beach, Virginia, area were made, sites of heavy C. hollensis breeding were not located. Small numbers of this species

emerged from several mud and sand samples taken near Rudee Inlet. The most productive breeding area found was a seepage or drainage ditch partially shaded by a sparse growth of Spartina alterniflora (Fig. 6). A thin layer of mud covering compacted sand apparently served as the larval substrate.

C. furens. - A number of larval habitats were located for this species at Virginia Beach. The site of greatest larval abundance, however, was essentially the same as that described for C. hollensis. The chief difference being that C. furens was found nearer the mouth of the ditch where sand and an accumulation of organic debris provided the larval substrate. There was little or no mud present and the area was possibly moistened, but not generally flooded, during high tide. S. alterniflora provided sparse cover and served to hold the accumulation of decaying organic debris.

C. melleus. - Adults of this species were by far the most abundant species at Virginia Beach during the summers of 1964 and 1965. However heavy concentrations of larvae were not found. Fifteen to 20 two-quart intertidal sand samples all yielded extremely small numbers of emerging adults. Most of these were females.

Fig. 6. A salt marsh breeding site of Culicoides hollensis (=canithorax) and C. furens.

Moist Terrestial Habitats

Mostly alkaline, dung-polluted, and generally exposed to direct sunlight. - Southwestern Virginia is a large cattle producing area and many overflowing watering troughs or small polluted streams in cow pastures provide excellent breeding sites for several species of Culicoides. Extreme pollution of an area seemed to limit the culicoid population to one, or possibly two species, whereas slight pollution seemed to provide a suitable habitat for several additional species.

C. crepuscularis. - This species was extremely abundant during the summer of 1964 and somewhat less in 1965. During 1964 several highly polluted watering areas provided sites for extensive larval breeding. An over-flowing watering trough at Chilhowie, Virginia (Smyth County) provided a larval breeding area 20-30 ft in diameter (Fig. 7). Mud, 2 ft deep in some areas, was covered with shallow pools of water. Light silt around the edges of these puddles produced the greatest numbers of larvae. The area was exposed to direct sunlight and was frequented by about 100 dairy cows. Massive numbers of C. variipennis variipennis were also present, as well as a few C. stellifer.

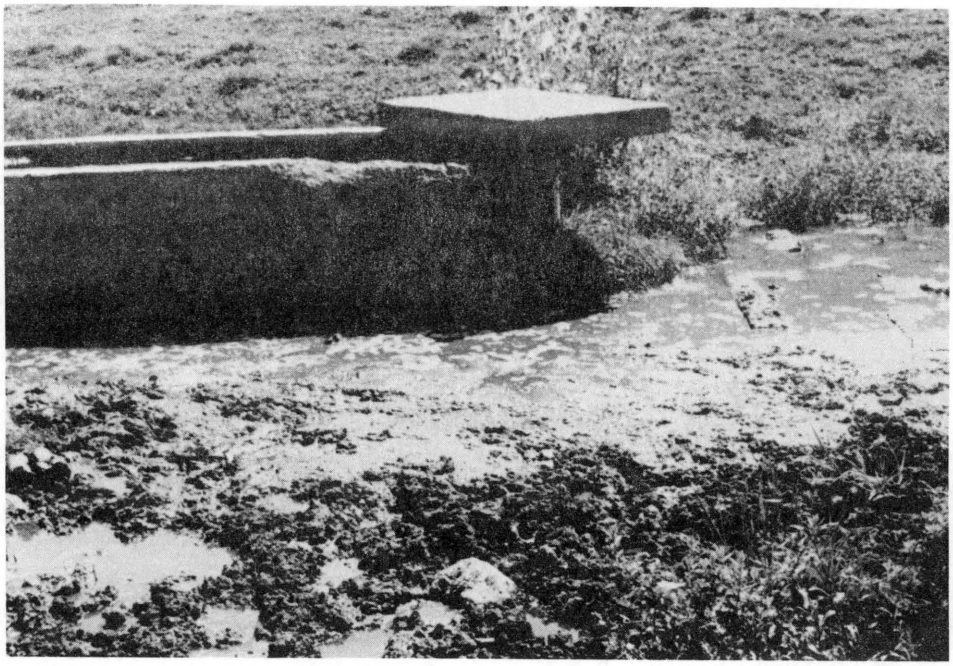

Fig. 7. Larval breeding site of Culicoides variipennis variipennis, C. crepuscularis and C. stellifer.

Another area of heavy breeding was a small watering pond near Blacksburg of approximately one-half acre in size which supplied water for about 150 dairy and beef cattle. Loose mud several feet wide and a foot or more deep bordered the small pond and was heavily polluted with cattle feces and urine. Mud and water from the area had an average pH of 9.5. C. variipennis variipennis were present in even greater numbers than C. crepuscularis.

A small, shallow, rocky stream flowing through a hog lot near Blacksburg provided an interesting breeding site for moderate numbers of C. crepuscularis and lesser numbers of C. haematopotus, C. variipennis variipennis and C. venustus (Fig. 8). The stream passed through a series of minute islands and pockets produced by frequenting hogs. Hog dung was very abundant and water from the still pockets had a pH of 9.2. The entire area was exposed to direct sunlight.

Numerous other animal watering areas supported small numbers of C. crepuscularis and all sites at which this species was found contained at least moderate pollution.

C. haematopotus. - Polluted sites as described for C. crepuscularis are probably not the most important breeding sites for C. haematopotus in Virginia, but since C. haematopotus was

Fig. 8. Polluted hog-lot stream which supported Culicoides crepuscularis, C. variipennis variipennis, C. haematopotus and C. venustus.

encountered several times at this type of habitat, it seemed necessary to comment on this type of environment as a secondary breeding site. In addition to the hog lot site mentioned under C. crepuscularis where C. haematopotus was found in very small numbers, two or three other very similar but less polluted habitats, produced small numbers of C. haematopotus, C. crepuscularis and C. v. variipennis.

C. obsoletus. - A pile of used chicken litter provided the only breeding site found for this species (Fig. 9). The litter contained about 60-75% wood shavings and the balance was chicken feces and feathers. The litter pile was located in an open field and received direct sunlight. The outer 2-3 inches of the pile was extremely dry and covered the slightly moist, decaying litter beneath. Most larvae were located at the moist-dry interface. Larvae were present in mid-August.

C. stellifer. - This species was reared from heavily polluted mud and water collected from an overflowing watering trough described under C. crepuscularis in this section.

C. variipennis variipennis. - This is an extremely abundant species in southwestern Virginia and several of the most productive habitats have been mentioned under the C. crepuscularis

Fig. 9. A pile of used chicken litter which provided a breeding site for Culicoides obsoletus.

heading of this section. Almost all areas having moisture and pollution from animal feces supported at least light C. v. variipennis breeding for most of the summer of 1964 and to a lesser degree in 1965. Habitats in central and southwestern Virginia were so numerous that it is impractical to mention all of them here. Most sites were alkaline. Northern and eastern sections of the state were not surveyed for this species.

C. venustus. - The only breeding site located for this species was the stream polluted by hog feces mentioned under the C. crepuscularis heading in this section.

Generally acidic, soil and plant habitats other than tree holes. - This heading is intended to cover a very broad range of habitats not listed under one of the other three habitat headings.

C. biguttatus. - A light population of C. biguttatus larvae were found to occupy the same habitat as C. stellifer, C. piliferus, C. spinosus and C. haematopotus near Vesuvius, Virginia, on a tributary of Big Mary's Creek. Areas of soft black mud partially covered with decaying leaves and bordering a small, swift flowing stream provided the larval substratum. The mud ranged up to 6 inches deep, was several feet wide in some areas and had a pH of 6.6.

Larvae also appeared on an occasional sand bar located within or near the edge of the stream. The area was heavily shaded but had very little undergrowth. Larvae were present from early July to September.

Two other habitats from Vesuvius included wet leaves from a shaded pool beside a small mountain stream and mud and decaying organic matter from the edge of the stream. In all cases C. biguttatus seemed to prefer extremely moist and shaded sites. The earliest larval collection was on June 18 and the latest on September 2, 1964.

C. guttipennis. - This species breeds almost exclusively in wet tree or stump-holes but one specimen emerged from wet leaves taken from a wildlife watering pond at the Broad Run Game Reserve in Craig County on July 12, 1964. A steep slope on one side of the pond accounted for an accumulation of leaves in that side of the small pond which had a pH of 6.7. The accumulation of leaves was several feet deep and partially submerged (Fig. 10). The area was shaded from afternoon sun by over-head vegetation.

C. haematopotus. - Several breeding sites for this species were located in this survey. Moderate numbers of larvae and pupae were removed from the wildlife watering pond in Broad Run Game

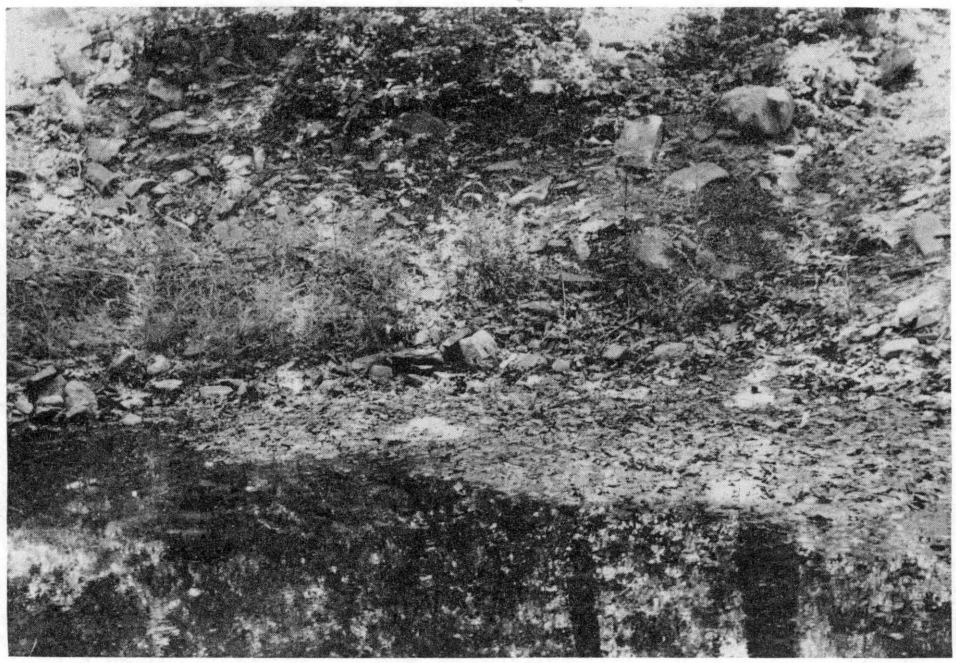

Fig. 10. Leaf accumulation in a watering pond edge which supported larvae of Culicoides guttipennis, C. haematopotus, C. sanguisuga and C. stellifer.

Reserve mentioned under C. guttipennis above. Larvae were present from early June until late July.

A sandy area on the bank of Big Mary's Creek between Mine and McLung Mountains in Rockbridge County also produced several C. haematopotus larvae and pupae. Mud and leaves from the edge of a tributary of Big Mary's Creek was a heavy breeding site for this species and supported small numbers of C. piliferus, C. spinosus, and C. stellifer.

The most extensive breeding area of C. haematopotus was located between Mine and McLung Mountains on Big Mary's Creek and has been described under the C. biguttatus heading of this section.

Mud from the edge of a half-acre wildlife watering pond in Broad Run Game Reserve was found to support small numbers of C. haematopotus. There was very little organic matter present and the larvae were found most abundantly very near the waters edge.

C. piliferus. - One C. piliferus larval habitat of soft black mud and decaying leaves has been listed under C. biguttatus in this section and mud and leaves from a creek's edge provided a second and is described under C. haematopotus. Small numbers of larvae were present at both sites.

C. sanguisuga. - The site at Broad Run Game Reserve in Craig County which was described for C. guttipennis supported small numbers of this species in mid-June 1964. In Rockbridge County, heavy breeding was encountered in mid-August of 1964 in a large pile of decaying leaves on the steep side of a road embankment between Mine and McLung Mountains. The outer few inches of the leaves in the pile were very dry and covered only slightly moistened leaves. C. sanguisuga larvae could be seen migrating between the leaves when compacted leaf packets were separated.

C. spinosus. - Moderate numbers of this species were recovered from the site of soft black mud with decaying leaves mentioned under C. biguttatus and the mud and leaf habitat referred to under C. haematopotus. Wet leaves from the side of a small intermittent stream at Broad Run Game Reserve in Craig County supported small numbers of C. spinosus. In all cases, breeding sites were found in well shaded areas.

C. stellifer. - This species was found breeding at several sites which have already been mentioned. The site which has been given for C. guttipennis in this section supported small numbers of larvae. Moderate numbers of C. stellifer larvae were found in the C. biguttatus and C. haematopotus habitats described above.

C. travisi. - Only one breeding site of this species was found. Small numbers of C. travisi were taken from wet leaves near the edge of a small stream at Broad Run Game Reserve in Craig County. The leaf accumulations had small amounts of mud and sand intermixed and were well shaded by overgrowth vegetation.

B. Culicoides guttipennis Rearing.

The methods and materials previously described have proven to be very satisfactory in the establishment of C. guttipennis in our laboratory. It is believed that at the current rate of production the colony can be maintained indefinitely without fear of loss. Jones (1960) has maintained C. variipennis sonorensis in a laboratory in Texas and Colorado for 9 years at essentially the same parent colony strength as we now have for C. guttipennis.

Some selection for a short larval period and long lived adults is being carried out. Most larvae over 3 weeks old are discarded and old adults are combined in a common cage and saved for egg production. The current adult production of our C. guttipennis colony is about 1000/day. Recent egg production has been so great that far more eggs are collected than needed to maintain the current status of the colony. Maintenance requires the services of a well-trained individual for 2-3 hours/day.

The generalized life cycle of C. guttipennis in the laboratory is about 25 ± 4 days - egg 2-3, larva 12+, pupa 3, prefeeding one or less, premating less than 2 days, preoviposition 4-5.

Diets. - The adult diet used has proven very successful and accounts for an average adult longevity of about 12 days. Both sexes feed readily on the liquid diets given in capillary tubes. The other constituents have also provided greater longevity.

The exact diet of larvae has not been fully determined but it is believed that detritus plays a very important part since larvae could not survive on mixed or pure cultures of soil bacteria isolated from the rearing chambers. Attempts to rear larvae on aseptic leaf mold failed due to the presence of microorganisms in the larval gut at the time of entrance. From our field and laboratory observations, it has been shown that larvae can be scavengers (i.e. feed on dead earthworms, dead insects or other organisms present as well as other organic matter), predators (i.e. feeding on live mosquito larvae of Orthopodomyia signifera (Coq.) and Aedes triseriatus (Say), or larvae of Helodidae (Coleoptera), or under extremely crowded conditions, cannibalistic. Their frequent habit of swimming slowly through algal and bacterial mats in laboratory rearing chambers might suggest that this too accounts for at least a portion of their

diet. For food in our laboratory rearing procedures, larvae are limited to bacterial and algal growths, microorganisms in the leaf mold, a few small stray invertebrate organisms in the leaf mold, and the leaf mold itself. Each 4-gal aquarium is generally capable of supporting about 1500 larvae.

Mating. - Temperature and humidity as well as proper lighting are especially important in stimulating mating. Mating is generally initiated while both sexes are in flight, but on occasions the male may dislodge a female from the side of the holding chamber and mating occurs as they fall to the floor of the cage. The conditions previously mentioned have proven successful in stimulating flight and mating. Excessive illumination causes uncoordinated flight and is unfruitful in stimulating mating. Newly engorged females are especially attractive to males.

Oviposition. - Oviposition occurs within the special oviposition vials along the air-water interface. The eggs are laid singly as the female slowly creeps along the filter paper in a fairly straight line near the water's edge. Dissections of gravid females reared in the laboratory show that they are capable of laying from 175-350 eggs in a single gonadotropic cycle.

Larval and pupal development. - With proper food each of the four larval instars is completed in about 3 days and pupation occurs after about 12 days. Adults emerge above the surface of the water from a dorsal pupal slit as the floating pupae reach 3 days of age. Surface scums are especially detrimental to pupae due to the need for atmospheric air by pupae and also scums interfere extensively with adult emergence. A slow stream of air from an aquarium pump alleviates this problem.

This venture at colonization of C. guttipennis has resulted in the laboratory establishment of a second species of Culicoides in the United States within the past 10 years. The two species have very diverse habitats, and probably habits, and should give us much information on the biology of the group as a whole. Techniques involved in rearing these two species should be applicable to a number of other species and lead to their successful establishment in the laboratory.

C. Host Preference Studies.

Table 4 shows the totals of all species of Culicoides collected in "Fallis and Bennett" type animal baited traps on 14 different hosts during the summer of 1965. Data from the box turtle, painted turtle and bull frog are omitted from the table since nothing was

Table 4. Totals of each species of Culicoides collected from different hosts in the summer of 1965 during 19 trapping nights.

Species Collected	Hosts Used											Accumulated Totals for all		
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	mallard	quail	dove	man	mammals	birds	man
arboricola	0	0	0	0	0	3	6	1	3	1	4	0	14	4
biguttatus	25	2	16	5	11	13	10	9	7	7	13	59	46	13
crepuscularis	3	0	5	0	3	12	17	11	6	6	8	11	52	8
furens	9	25	13	*	5	20	*	6	*	*	70	52	26	70
guttipennis	36	11	107	10	75	8	14	12	3	2	130	239	39	130
haematopotus	12	6	17	4	12	21	34	22	7	5	39	51	89	39
hinmani	2	0	11	0	13	0	0	0	0	0	27	26	0	27
hollensis	0	0	0	*	0	0	*	0	*	*	50	0	0	50
melleus	13	3	3	*	0	2	*	4	*	*	200	19	6	200
parensis	0	0	0	0	0	0	0	0	0	0	8	0	0	8
sanguisuga	369	119	222	50	226	46	58	40	11	6	498	986	161	498
stellifer	13	2	1	2	0	1	3	0	0	0	3	18	4	3
travisi	0	6	5	0	2	8	5	12	8	3	3	13	36	3
variipennis	0	0	0	0	0	0	0	0	0	0	3	0	0	3
villosipennis	0	0	0	0	0	0	0	0	0	0	1	0	0	1

*Host not used in breeding area of this species.

collected on any of these hosts during the trappings. These totals represent 19 trapping nights and the pooling of several trapping periods on some nights. Field data appear in the Appendix. Table 4 also includes accumulated totals numbers of each species of Culicoides collected on mammals as a group, birds as a group, and man. It was thought that accumulated totals might give an indication as to the broad host preferences -- that is, ornithophilic, mammalophilic, or man, whereas some individual observations on mammals, birds and man might not differ greatly. Since Bennett (1960) concluded that certain species are fairly specific as to host, perhaps this is not a good idea.

Due to excessive variation (i.e. sites, seasonal variation of Culicoides, weather, etc.) and small numbers to work with, the data could not be analyzed statistically. However, it is felt that certain totals are possibly biologically significant. In the accumulated totals of Table 4, it is interesting to note the absence of C. arboricola on small mammals. The four specimens collected on man represent the first records of this species feeding on man (Jamnback 1965). C. biguttatus, C. furens, and C. haematopotus appear to be more general feeders but some slight preference is shown in some cases. C. stellifer also probably falls in this category. C. crepuscularis has most generally been recorded as ornithophilic and our limited

data tend to support this contention. C. hollensis and C. melleus have never been reported on hosts other than man but the few C. melleus collected on other hosts in these tests is an indication that other hosts can be involved. It is apparent that man is the preferred host of these two species. C. parensis is noted as a diurnal feeder and has been collected extensively on man. The eight specimens collected in Virginia were all on man. C. variipennis has infrequently been recorded on man but seems to prefer the larger mammals (Wirth and Jones 1957). The three specimens taken in this study were collected in areas of heavy variipennis breeding. C. sanguisuga has been recorded biting a variety of different hosts and is an especially vicious pest of man. Our experimental data would tend to show that it prefers mammals and man over birds as hosts. C. guttipennis has previously been recorded feeding on man and other large mammals. Although Jamnback (1965) lists it as probably an ornithophilic species, our data indicate that small mammals are probably the preferred hosts and that man is also very susceptible to attack. It is interesting to note that C. guttipennis was collected most frequently on the smaller of the small mammals. C. travisi was collected most abundantly on birds. Jamnback (1965) proposed that this species was a possible ornithophilic species based on the number of sensory pits on the antennal segments.

The data collected in these preliminary tests are so superficial that no definite conclusions can be made on host preferences. Several years' data from extensive trapping will probably be necessary before any meaningful data can be accumulated. The bionomics of this group of flies should be carefully considered prior to designing the experiments and experiments set up so that statistical analysis of the data would be possible.

D. Culicoides Activity Studies.

Table 5 presents the limited data collected in the activity studies. Dr. C. Y. Kramer of the Statistics Department of Virginia Polytechnic Institute felt that there were insufficient data to make a statistical analysis. In general, most species appeared more abundantly in the 9-12 PM and 12-3 AM collections. C. biguttatus was collected in largest numbers in the early morning hours between 3 and 6 AM. Since the preliminary data are fairly consistent this might indicate the period of greatest activity. C. haematopotus, C. stellifer and C. venustus are the only other species with sufficient numbers to give an indication as to the probable periods of activity. C. haematopotus appears to be almost equally as active during the 9-12 PM and 12-3 AM periods, whereas C. stellifera and C. venustus activity seems to be higher in the earlier night hours and decreases as the night progresses.

Table 5. Nocturnal activity of Culicoides during the summer of 1965 as indicated by light traps at Blacksburg, Virginia.

Species	No. collected during indicated period*		
	9-12 PM	12-3 AM	3-6 AM
arboricola	3	0	0
baueri	1	0	0
biguttatus	33	45	73
crepuscularis	11	12	1
guttipennis	14	9	6
haematopotus	85	79	39
sanguisuga	19	37	11
stellifer	79	34	21
travisi	5	4	9
variipennis	10	13	5
venustus	37	15	6
villosipennis	1	0	0

*Total from 11 trapping nights.

V. DISCUSSION AND CONCLUSIONS

In general the breeding sites of Culicoides in Virginia closely paralleled reports of breeding areas in other sections of the United States. The collecting of C. footei was considered a very important find since this species is so rare. C. stellifer has never been collected from hollow trees or other living plant environments (Wirth 1965) even though several workers have misquoted Wirth (1952) as having recovered this species from tree wound ooze. This finding can probably be considered a new breeding site record for this species. C. sanguisuga is seldom found breeding in wet hollow stumps as was discovered once in this study. Those species of Culicoides recovered in the saline habitats were expected to be present since many other workers have reported on similar habitats for these species. The only other unusual breeding site of Culicoides found in this study was an accumulation of leaves in a wildlife watering pond which supported C. guttipennis. This species normally breeds in wet tree and stump-holes.

Even though some other improvements on rearing of C. guttipennis can surely be made, the present methods are producing vigorous adults in a very reasonable length of time. It is felt that

one of the most important factors in these colonization procedures was to supply the proper illumination, humidity and temperature to induce mating and to reduce adult mortality.

These rearing procedures can possible be applied to other wet tree-hole breeding Culicoides and result in successful establishment of other colonies of this genus.

Since the host preference data in these tests do not show decided differences in specific host preferences (i.e. rabbit over guinea pig) as did some of Bennett's work (1960) on ornithophilic hosts, it is felt that more preliminary work is needed on the ranges of hosts of Culicoides. That is, the range of mammalophilic feeding species and ornithophilic feeders. One might conclude on the basis of the limited data collected in these tests that many species readily attack any number of hosts. The data collected by Bennett (1960) seem to infer more specific feeding habits of most species. A timely and extensive study is called for before definite conclusions on host preferences can be made. It is felt that far too little is known about the feeding habits of most species even to place them in one of the brood categories (i.e. ornithophilic.)

The scant data obtained in the adult activity studies can probably be attributed to the poor selection of the trapping site. However Messersmith (1961, 1964) found the area to be most

productive in numbers and a variety of species. The time-operated traps worked very well in these studies and should provide valuable data through further use.

VI. SUMMARY

Culicoides have been recognized for many years as vicious pests of man in all parts of the world. Not only do these minute bloodsuckers cause severe skin irritation and produce allergenic dermatitis in some livestock and man, but they serve as vectors for a number of diseases, especially filariasis.

Partially due to the minuteness and difficulty with which these small flies are handled, they have grossly been neglected as potential vectors of diseases. When considering the group as a whole, practically nothing is known about the bionomics of Culicoides. Work in Virginia on Culicoides prior to this study has been limited primarily to adult surveys.

This study was undertaken in the summer of 1964 to investigate some areas of the bionomics of Virginia Culicoides through field and laboratory studies. Laboratory colonization attempts were undertaken so that detailed information might be obtained on any species successfully colonized. A larval survey was undertaken so that breeding sites could be determined. Preliminary host preference studies were initiated so that a successful animal-baited trap could be found and so that preliminary data on host preferences could be collected. In order to learn more about periods of adult activity,

time-switch operated New Jersey light traps were set to operate at different time periods during each trapping night.

Laboratory colonization attempts with C. guttipennis has led to the successful establishment of this species in the laboratory. The colony has now completed over 15 generations since its establishment 1 year ago and is currently producing an average of more than 1,000 adults per day. Some information on the laboratory biology has been obtained and future work is planned.

One or more breeding sites was located and described for 22 of the 32 species of Culicoides known to exist in Virginia. In host preference studies, four types of animal-baited traps were given preliminary testing and only one proved to be a satisfactory survey tool. During 19 trapping nights 15 species of Culicoides were collected on 1 or more of 14 different hosts used. Even though some preferences are possibly shown in the limited data collected, much more data will be required before definite conclusions can be drawn.

More data will also be required before periods of activity for most species can be accurately given. Only a few species were collected in great enough numbers to allow "educated guesses" as to the probable periods of activity in this area.

This preliminary work will provide a basis on which further investigations in several areas of bionomics of Culicoides can be

conducted over the next 3 years. The proposed future work, to be supported by the U. S. Department of Agriculture, should give more conclusive data on many phases of the bionomics of Culicoides.

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VIII. APPENDIX

Table I. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia, On July 25, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
guttipennis	0	0	18^{12}	2	9^{6}	2^{2}	2^{2}	0	0	0	0	0	1^{1}	7
hinmani	0	0	4^{2}	0	3^{1}	0	0	0	0	0	0	0	0	3
parensis	0	0	0	0	0	0	0	0	0	0	0	0	0	3
sanguisuga	14^{7}	4^{2}	4^{3}	1	9^{4}	3	2	0	1	0	0	0	6^{3}	19
travisi	0	0	0	0	0	0	0	0	1	0	0	0	0	1

Table II. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia on August 2, 1965.** Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	0	*	1	*	1	0	0	*	*	*	*	*	*	3
guttipennis	6^{3}	*	23^{14}	*	0	0	0	*	*	*	*	*	*	12
hinmani	2^{1}	*	4^{3}	*	3^{2}	0	0	*	*	*	*	*	*	14
sanguisuga	19^{7}	*	26^{15}	*	1^{1}	0	0	*	*	*	*	*	*	32
stellifer	0	*	0	*	0	0	0	*	*	*	*	*	*	2
villosipennis	0	*	0	*	0	0	0	*	*	*	*	*	*	1

*Host not used.

**Composite of three trapping periods.

Table III. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia on August 9, 1965.** Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	1	0	0	*	0	0	1	*	*	*	*	*	*	0
guttipennis	5^{2}	0	6^{2}	*	1^{1}	0	0	*	*	*	*	*	*	3
sanguisuga	90^{32}	4^{2}	19^{12}	*	4^{1}	7^{3}	12^{6}	*	*	*	*	*	*	45
villosipennis	0	0	1	*	0	0	0	*	*	*	*	*	*	0

*Host not used.

**Composite of three trapping periods.

Table IV. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia on August 26, 1965.* Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
arboricola	0	0	0	0	0	2^{1}	5^{3}	2^{1}	1	0	0	0	1^{1}	0
biguttatus	3^{3}	1	2^{1}	0	1^{1}	3^{1}	1	1^{1}	0	0	0	0	1^{1}	1
guttipennis	3^{2}	3^{2}	3^{2}	1^{1}	8^{6}	2^{1}	2^{1}	1	1	0	0	0	2^{1}	9
haematopotus	6^{3}	2^{1}	4^{4}	3^{2}	5^{5}	2^{1}	5^{3}	2^{1}	1	0	0	0	3^{2}	11
hinmani	0	0	2^{2}	0	1	0	0	0	0	0	0	0	0	1
parensis	0	0	0	0	0	0	0	0	0	0	0	0	0	2
sanguisuga	21^{8}	10^{7}	17^{9}	3^{2}	11^{8}	3^{1}	2^{1}	1^{1}	1	0	0	0	3^{2}	32

* Composite of two trapping periods.

Table V. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia in August 1965. Superscript indicates number engorged.

Species Collected	Hosts Used														
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
						August 27**								
arboricola	0	0	0	0	0	1^{1}	0	*	0	*	*	*	0	2
guttipennis	2	0	0	0	0	0	0	*	0	*	*	*	2^{1}	13
sanguisuga	1	0	1	1^{1}	0	0	0	*	0	*	*	*	0	16
						August 31								
guttipennis	0	0	1	0	1^{1}	0	0	0	0	0	0	0	0	2
haematopotus	1	0	0	0	0	0	1^{1}	0	0	0	0	0	0	1
sanguisuga	3^{2}	0	4^{2}	0	0	2^{1}	1	1^{1}	0	0	0	0	0	5

*Host not used.

**Composite of two trapping periods.

Table VI. Culicoides collected in animal-baited traps on various hosts at New Castle, Virginia on September 2, 1965.** Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
arboricola	0	0	0	0	0	0	1^{1}	1^{1}	0	*	*	*	0	2
guttipennis	7^{4}	0	29^{15}	0	29^{15}	1^{1}	2^{1}	2^{2}	0	*	*	*	2^{1}	44
hinmani	0	0	1^{1}	0	4^{3}	0	0	0	0	*	*	*	0	5
sanguisuga	16^{5}	0	2	0	0	2	1	0	0	*	*	*	1	14
parensis	0	0	0	0	0	0	0	0	0	*	*	*	0	2

*Host not used.

**Composite of two trapping periods.

Table VII. Culicoides collected in animal-baited traps on various hosts at Poly Scientific pond, Newport, Virginia, on July 20, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	3^{2}	1	2^{1}	0	4^{2}	1^{1}	1	0	1^{1}	0	0	0	0	1
guttipennis	1	0	4^{3}	0	1	0	1^{1}	0	0	0	0	0	1	8
sanguisuga	24^{11}	18^{12}	16^{9}	1	8^{4}	1	6	0	0	0	0	0	3^{2}	19
stellifer	6^{3}	0	1^{1}	2^{1}	0	0	1^{1}	0	0	0	0	0	0	0
travisi	0	6^{3}	2^{1}	3^{3}	0	2^{2}	3^{3}	1^{1}	0	0	0	0	5^{4}	0

Table VIII. Culicoides collected in animal-baited traps on various hosts at Poly Scientific pond, Newport, Virginia, on July 21, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	3^{1}	0	1^{1}	2^{1}	0	3^{3}	1^{1}	0	0	0	0	0	2	1
guttipennis	0	0	3^{2}	0	4^{1}	0	2^{2}	0	0	0	0	0	0	9
sanguisuga	29^{20}	7^{4}	4^{2}	8^{4}	19^{4}	3^{2}	6^{2}	1^{1}	0	0	0	0	8^{4}	34
stellifer	5^{3}	2^{1}	0	0	0	1	2^{2}	0	0	0	0	0	0	0
travisi	0	0	2^{1}	0	0	1^{1}	0	4^{1}	2^{2}	0	0	0	0	1

Table IX. Culicoides collected in animal-baited traps on various hosts at Poly Scientific pond, Newport, Virginia, on August 10, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	2^{1}	0	5	0	1	0	0	2^{1}	0	0	0	0	0	0
haematopotus	0	0	2^{2}	0	3^{3}	0	2^{2}	0	0	0	0	0	0	1
sanguisuga	12^{6}	5^{3}	17^{9}	12^{9}	7^{4}	0	0	2^{1}	0	0	0	0	4^{2}	26
stellifer	0	0	0	0	0	0	0	0	0	0	0	0	0	1
travisi	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table X. Culicoides collected in animal-baited traps on various hosts at Poly Scientific pond, Newport, Virginia, on August 16, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	0	0	0	0	0	3^{1}	3^{2}	0	1^{1}	0	0	0	0	3
crepuscularis	0	0	0	0	0	2^{1}	3^{2}	0	1^{1}	0	0	0	3^{3}	4
guttipennis	2^{1}	6^{4}	3^{2}	1	4^{3}	1^{1}	0	0	0	0	0	0	0	3
sanguisuga	4^{2}	21^{16}	19^{9}	3^{3}	21^{12}	6^{3}	1	2^{2}	2^{2}	0	0	0	3^{3}	21
travisi	0	0	0	0	0	2^{1}	0	1^{1}	0	0	0	0	5^{1}	0

Table XI. Culicoides collected in animal-baited traps on various hosts at Poverty Creek, Newport, Virginia, on August 18, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
crepuscularis	0	0	2	0	0	1	2	2	0	0	0	0	3	0
guttipennis	4	1	9	3	9	0	2	0	0	0	0	0	0	6
haematopotus	0	0	0	0	0	2	6	1	0	0	0	0	4	3
hinmani	0	0	0	0	2	0	0	0	0	0	0	0	0	1
sanguisuga	10	19	8	3	26	5	6	0	1	0	0	0	3	42
variipennis	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table XII. Culicoides collected in animal-baited traps on various hosts at Poverty Creek, Newport, Virginia, on August 24, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
crepuscularis	2^1	0	1^1	0	3^3	4^2	9^6	2^2	3^2	0	0	*	5^4	3
guttipennis	2^2	0	5^3	2^1	4^4	1^1	1^1	0	0	0	0	*	3^1	6
haematopotus	3^1	1^1	5^4	1^1	3^1	3^2	7^3	2^1	2	0	0	*	6^3	0
hinmani	0	0	0	0	0	0	0	0	0	0	0	*	0	3
sanguisuga	13^4	5^3	14^9	5^4	11^7	3^3	3^1	1^1	0	0	0	*	2^2	24
parensis	0	0	0	0	0	0	0	0	0	0	0	*	0	1
variipennis	0	0	0	0	0	0	0	0	0	0	0	*	0	1

*Host not used.

Table XIII. Culicoides collected in animal-baited traps on various hosts at Poverty Creek, Newport, Virginia, on September 6, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
sanguisuga	1^1	2^1	0	1^1	3^1	0	0	*	*	0	0	0	0	4

*Host not used.

Table XIV. Culicoides collected in animal-baited traps on various hosts in an upland wooded area, Blacksburg, Virginia, on August 23, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	2^{1}	0	4^{3}	0	1^{1}	3^{1}	2^{2}	4^{3}	3^{2}	0	0	0	6^{3}	0
crepuscularis	1	0	2^{2}	0	0	4	3^{1}	2^{2}	2^{1}	0	0	0	3^{2}	2
guttipennis	2^{1}	1^{1}	3^{3}	1	5^{4}	1	2^{1}	0	1	0	0	0	1^{1}	8
haematopotus	2^{2}	3^{3}	5^{4}	0	1^{1}	6^{3}	3^{2}	1^{1}	2^{1}	0	0	0	6^{3}	9
sanguisuga	9^{5}	6^{4}	11^{8}	3^{2}	8^{8}	2^{1}	3^{2}	1	0	0	0	0	2^{2}	18
travisi	0	0	1	0	2^{1}	3^{3}	2^{1}	2^{2}	1^{1}	0	0	0	2^{1}	0
variipennis	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table XV. Culicoides collected in animal-baited traps on various hosts in an upland wooded area, Blacksburg, Virginia, on September 11, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	0	0	0	0	0	0	1	0	0	0	0	0	0	0
sanguisuga	3^{1}	0	1^{1}	0	3^{1}	0	0	0	0	0	0	0	1^{1}	3

Table XVI. Culicoides collected from various hosts in animal-baited traps at Cranberry Glades, West Virginia, on July 29, 1965. Superscript indicates number engorged.*

Species Collected	Hosts Used					
	tame rabbit	guinea pig	white rat	chicken	turkey	man
biguttatus	10^{4}	1	3	0	0	1
guttipennis	2^{1}	0	0	0	0	0
sanguisuga	74^{45}	31^{11}	71^{33}	1^{1}	3^{1}	99

Table XVII. Culicoides collected in animal-baited traps on various hosts at Vesuvius, Virginia, on July 26, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used													
	tame rabbit	wild rabbit	guinea pig	opposum	white rat	chicken	turkey	quail	dove	box turtle	painted turtle	bull frog	mallard duck	man
biguttatus	1^{1}	0	0	3^{1}	0	0	0	0	2	0	0	0	0	3
haematopotus	0	0	1^{1}	0	0	8^{2}	10^{8}	1^{1}	0	0	0	0	3^{1}	14
sanguisuga	26^{18}	18^{6}	28^{16}	9^{3}	22^{18}	9^{4}	12^{8}	3^{2}	1	0	0	0	4^{3}	100
stellifer	2^{1}	0	0	0	0	0	0	0	0	0	0	0	0	0

Table XVIII. Culicoides collected in animal-baited traps on various hosts at Rudee Inlet, Virginia Beach, Virginia, on August 12, 1965. Superscript indicates number engorged.

Species Collected	Hosts Used									
	tame rabbit	wild rabbit	guinea pig	white rat	chicken	mallard duck	bull frog	box turtle	painted turtle	man
furens	9^{1}	25^{2}	13^{1}	5	20^{1}	6^{1}	0	0	0	70
hollensis	0	0	0	0	0	0	0	0	0	50
melleus	13^{8}	3^{2}	3^{2}	0	2	4^{2}	0	0	0	200+

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X. ABSTRACT

Several areas of the bionomics of Culicoides in Virginia were given preliminary investigation. These included surveys for larval habitats, colonization attempts with Culicoides guttipennis (Coq.), host preference studies and adult activity studies. Breeding sites of 22 species of Culicoides were located and briefly described. Samples of mud, debris, etc. from suspected breeding sites were brought into the laboratory and maintained at 70°F in food containers with screened tops. Most species were found to prefer a specific type of habitat (i. e. tree-holes, polluted areas, etc.) but exceptions were occasionally observed.

Colonization attempts with C. guttipennis have resulted in the successful establishment of this species in the laboratory. The colony has been maintained for 12-15 generations and is currently producing over 1,000 adults per day and an excess of eggs. Techniques developed for larval and adult maintenance have proved to be highly successful in recent months. Adults were held in a constant temperature cabinet at 80°F ± 2° and 85% relative humidity ± 10% in semi-darkness (less than 1 ft-c). Eggs were collected on moist filter paper exposed in shell vials. Fly eggs hatched in 3 days and the young larvae were introduced into aquaria containing leaf mold from

hardwood forests and distilled water. Larvae pupated in about 12 days and adults emerged about 3 days later.

The adult diet consisted of rabbit blood, raisins, sugar cubes, honey water and a 6:6:1:13 mixture of powdered milk, sugar, egg solids and water administered in capillary tubes.

In host preference studies 15 species of Culicoides were collected on 1 or more of 14 different hosts used in animal-baited traps. Animals were restrained and exposed on a small platform. After 15 min the animals were covered with a collecting cage. Flies were aspirated from the traps and killed in 70% ethanol. Even though some preferences possibly were shown by some species, more data will be needed before definite conclusions can be drawn.

In adult activity studies, most species appeared to be more abundant from 9 PM to 3 AM as indicated by light traps. This was especially noted in C. stellifer, C. haematopotus and C. venustus. The activity of C. haematopotus was fairly constant up to 3 AM and then decreased. C. stellifer and C. venustus activity decreased as the night progressed.