STUDIES ON CULICOIDES (DIPTERA: CERATOPOGONIDAE) AND THEIR RELATIONSHIP TO INFECTIOUS SYNOVITIS IN POULTRY IN VIRGINIA

by

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II. INTRODUCTION

Reports of increasing incidence of infectious synovitis in Virginia since about 1953 have led personnel at the Virginia Polytechnic Institute to an interest in the role of biting insects as possible vectors of the disease. This interest eventually resulted in a study of the role of bloodsucking Diptera in the transmission of the disease, supported in part by a grant from the National Institutes of Health.

Certain characteristics of infectious synovitis in poultry made Bloodsucking Diptera, especially Culicidae (mosquitoes) and Ceratopogonidae (biting midges), under suspect as vectors. Indeed, this has been remarked upon by Hinshaw and McNeil (1952) who reported a decrease in incidence of synovitis following mosquito control in a turkey area. The presence of the agent in the blood of chickens as reported by Olson et al. (1956 and 1957) and its distribution to almost all body tissues as shown by Cover and Benton (1957) would seem to indicate the possibility of a bloodsucking dipterous vector.

Culicoides (biting midges) are known vectors of certain tropical filaria as reported by Sharp (1927, 1928) and most recently by Hopkins and Nicholas (1952). However, of even more significance is the fact that they have been shown by DuToit (1944) to transmit the virus of blue-tongue disease in sheep in South Africa and in Texas by Price and Hardy (1954). Also of interest to our work is the fact that Tokunaga (1937) showed Culicoides to be a vector of fowlpox.

In addition it is known that several species of Culicoides are avian feeders, as shown by Judd (1957) and Fallis and Wood (1957), who also have shown them to be intermediate hosts to Haematoproteus (Protozoa) of ducks. A number of species of Culicoides have been collected in poultry houses by Snow et al. (1957), Wirth and Bottimer (1956) and others. Many of these were engorged females. Hence it was felt that a study of this genus of insects as possible vectors of infectious synovitis was justified.

During the early stages of these investigations it was found that only a little information was available on Culicoides in Virginia. Murray (1957) had investigated biting midges at Mt. Solon, Snow et al. (1957) had noted their abundance at Saltville, and Wirth (1951) remarked upon their abundance at Mt. Solon, and Falls Church. Some other authors, especially Foote and Pratt (1954), reported additional locality records. Thus it was felt that much could be learned about Culicoides per se in Virginia while investigating their role as vectors.

Therefore, the objectives of this study are as follows:

1. To collect and identify the Culicoides species indigenous to Virginia.
2. To study the bionomics and life histories of Virginia Culicoides.
3. To attempt to ascertain which species of Virginia Culicoides are associated with poultry.

4. To develop improved methods of laboratory rearing of Culicoides for vector studies.
5. To investigate Culicoides as a possible natural vector of infectious synovitis.
6. To attempt to determine if a viremia exists and the length of time it remains viable and transmissible by Culicoides in the laboratory.

III. REVIEW OF LITERATURE

Culicoides as Vectors

Culicoides were suggested as possible vectors of tropical fevers by Kinoshita (1918), Loughnan (1921), Stephens (1923), Purcell (1937), Wanson (1939), and Muttel et al. (1953). Sharp (1927, 1928) was the first to show that filaria were transmitted by Culicoides. He carried on his investigations in Africa. Buckley (1938) found them to be vectors of onchocerciasis in horses and cattle in Malaya. Others who have concurred with his findings are Steward (1933, 1935), Moignoux (1951), and Wehr and Lucker (1952). However, Gibson and Ascoli (1952), as reported in Wirth (1955), found no Onchocerca volvulus in wild-caught Culicoides in an onchocerciasis area of Guatemala.

The pathogenic agents of other animal diseases transmitted by Culicoides are those of African horse sickness and blue tongue of sheep as reported by DuToit (1944, 1945). Price and Hardy (1954) reported similar findings in Texas in sheep as did Bradley (1954). Fowlpox was shown by Tokunaga (1937) to be transmitted by Culicoides in Formosa, as was equine encephalitis in Ecuador by Levi-Castillo in Karstad et al. (1957).

Unidentified filaria have been found in Culicoides by Sergent et al. (1933), Dampf (1935, 1936a, 1936b) Causey (1938), Hoffman (1939), Vargas (1941), and Mirsa et al. (1952). Desportes (1941) reported a filaria in frogs transmitted by Culicoides. Due to

finding engorged female Culicoides in nests of birds infected with filaria, Jellison (1940) and Robinson (1955) suspected Culicoides as vectors of avian filaria.

In Canada, Haematoproteus (Protozoa), a blood-parasite of birds, has been shown to be transmitted by Culicoides as reported by Fallis and Wood (1957), Fallis (1958), Bennett and Fallis (1960), and Fallis and Bennett (1960).

Culicoides have also been proven to be vectors of three filaria found in humans: Acanthocheilonema perstans as reported by Sharp (1927, 1928), Roman (1941), Garnham and Harper (1944), Kershaw (1950), Hopkins (1952), Nicholas et al. (1952), Hopkins and Nicholas (1952), Nicholas (1953a), Nicholas et al. (1953), and Nicholas and Kershaw (1954); Acanthocheilonema streptocerca by Chardome and Peel (1949), Henrard and Peel (1949), Van Den Berghe and Chardome (1952), and Duke (1954, 1956); and Mansonella ozzardi by Buckley (1933, 1934a, 1934b), O'Connor (1937), Briceño-Rossi (1949), and Romaña and Wygodzinsky (1950).

Feeding Habits

The feeding habits of these minute flies have been a source of annoyance to man in all parts of the world, especially in areas near the water as reported by Séguy (1950). In our own country their annoyance caused the Creek Indians to place smudge pots under their

beds to drive them away according to Swanton (1922), while Pratt (1907) referred to their "cussedness" in mountainous areas in Virginia. Their biting has driven tourists from coastal resorts, and has even led to the capture of an escaped convict according to Dove et al. (1932). They have caused great annoyance to Panama Canal personnel as described by Carpenter (1951), Woke (1954) and Blanton et al. (1955), and to girl scouts in a camp at Mt. Solon, Virginia, according to Murray (1957).

Not only is their biting of great annoyance to man, but many persons have severe dermal reactions as illustrated by Jobling (1928), Hase (1933), Carpenter (1951), Edmunds and Keener (1954), Blanton et al. (1955) and Vargas (1960). The histopathic changes in the skin of a man who suffered a severe reaction to the bite of C. furens were studied by Aréan and Fox (1955).

Among the species of Culicoides indigenous to the United States are some that are nocturnal and some that are diurnal biters. Reports of the nocturnal species have been published by Snow and Pickard (1953) in Tennessee, Edmunds and Keener (1954) in Nebraska, Williams (1955) in Michigan, Murray (1957) in Virginia and Snow et al. (1958) in South Carolina. Diurnal biters have been reported by Williams (1955), Snow and Pickard (1953), Pickard and Snow (1955), Snow (1955), Murray (1957), and Snow et al. (1957, 1958).

The vertical distribution in trees of biting species of Culicoides has been studied by Snow (1955), Williams (1955), Snow et. al. (1958)

and Bennett (1960). They found that certain biting species are more common at certain heights and that some species rise with decreasing light.

Besides feeding on man they have been discovered feeding on a number of other victims or nutriments. Some of these references are: Culicoides feeding on horses in England by Steward (1933), in South Africa by DuToit (1944), in Panama by Carpenter (1951), in the United States by Pickard and Snow (1955), in Australia by Lee (1956) and in England by Campbell and Pelham-Clinton (1960); feeding on cattle by Leuckart (1893), Smith and Swaminath (1932), in Malaya by Buckley (1938), in England by Hill (1947), in Australia by Lee (1956), in the United States by Jones (1959) and in England by Campbell and Pelham-Clinton (1960); feeding on sheep in South Africa by DuToit (1944), in the United States by Price and Hardy (1954) and Jones (1959), and in Australia by Lee (1956); feeding on goats and pigs in England by Campbell and Pelham-Clinton (1960); feeding on monkeys by Causey (1938); on rabbits in Australia by Lee (1956) and in the United States by Jones (1958, 1959); and feeding on mice by Jones (1959). Reports of Culicoides feeding on wild birds have been published by Painter (1926), Arnaud (1956), and Hicks (1959); on crows by Jellison and Philip (1933); on catbirds by Judd (1954, 1957); on house finches by Wirth and Hubert (1960) and Ryckman (1960); on spruce grouse and ruffed grouse by Fallis and Bennett (1960); and on 11 different birds by Bennett (1960). Examples of Culicoides feeding

on domestic birds such as chickens, turkeys and unidentified fowls have been given by Tokunaga (1937), Jones (1954), Snow (1957), Pickard and Snow (1955), Lee (1956), Arnaud (1956), Snow et al. (1957, 1958), Jones (1959), and Campbell and Pelham-Clinton (1960). Accounts of Culicoides feeding on dogs, flying foxes, and marsupials have been published by Lee (1956); of Culicoides feeding on turtles (Clemys insculpta) attributed to Wirth (in litt.) by Downes (1958); feeding on lizards by Myers (1935); and feeding on engorged mosquitoes by Edwards (1922), Sinton and Little (1925) and Séguy (1950).

A report by Patel (1921), and Hill (1947), that Culicoides feed on earthworms is said by Downes (1958) to be incorrect. However, they do feed on flowers either as their sole source of food or as a supplemant to a blood meal (Kieffer, 1925; Mayer, 1933; Downes, 1955, 1958; and Becker, 1960.)

Downes (1950) made the useful observation that C. nubeculosus in captivity will not feed until two or three days after emergence as adults.

C. variipennis, the species used in most of our transmission studies, has been reported feeding on man by Knowlton and Fronk (1950), Pickard and Snow (1955), Snow et al. (1957) and Jones (1959). They also have been observed under laboratory conditions to feed on cattle, sheep, rabbits, mice and a chicken by Jones (1959), while Price and Hardy (1954) reported their feeding on sheep during their blue-tongue studies.

Trapping

While searching for the breeding places of Culicoides in Atlantic coastal marshes Dove et al. (1932) utilized a recovery cage that trapped adults as they emerged from the water. A similar device has been utilized in Alaska by Sailer et al. (1956), Williams (1956) and Wirth and Williams (1957). Horse-baited traps have been employed by Gluchova (1958) in Karelia, USSR, and in Panama by Carpenter (1951). Bennett (1960) used bird-baited traps.

James (1943), while making a survey of Culicoides in northern Colorado, was the first to use light traps to capture these minute insects. Since that time the New Jersey type light trap has been employed by many workers throughout the world. For instance, in Australia Lee (1956) and his co-workers have done extensive light-trapping. In Bermuda much of the survey work of Williams (1956) and Wirth and Williams (1957) utilized light traps. In Canada Downes (1958) used them. They have been used in Japan and neighboring areas by Tokunaga (1955) and Arnaud (1956). Hill (1947) and Campbell and Pelham-Clinton (1960) used light traps in England. In their onchocerciasis studies in Guatemala, Gibson and Ascoli (1952) collected Culicoides with light traps. The Panama work of Wirth and Blanton (1953, 1955a, 1955b, 1956a, 1956b, 1959) was done by light trapping as was Carpenter's Panamanian work in 1951. Puerto Rican survey work utilized light traps as reported by Fox and Capriles (1953), Fox (1949, 1953) and Fox and Kohler (1950); and finally, DuToit (1944) used a light trap modified in such a way that the flies did not pass through the fan when he did his research in South Africa.

In the United States several workers have made use of light traps while studying Culicoides. Jones, Butler and Gloyd used light traps to collect cactiphilic species in the west as reported by Wirth and Hubert (1960). They were used in California to collect all types of aquatic insects according to Lattin in Usinger (1956). Lewis (1959) used them in his research in Connecticut. They were used at various places in New England as reported by Coher et al. (1955). Light traps in Florida helped Beck (1951, 1952, 1956, 1958) in her studies of that state's Culicoides population. Williams (1955a, 1955b) made use of light traps in Georgia and Michigan. The Culicoides of western Nebraska were collected at light traps by Edmunds and Keener (1954). In the Tennessee River Basin, a great deal of light-trapping was done by Snow and Pickard (1953), Pickard and Snow (1955) and Snow et al. (1957). Studies in Texas were assisted by light trapping as mentioned in Price and Hardy (1954), Wirth and Bottimer (1956) and Jones and Wirth (1958). At Mount Solon, Virginia, Murray (1957) employed light traps as did Bacon and Pullman (1953) in the Columbia Basin in Washington.

Most of the work on the relative attractiveness of differently colored lights to insects has been conducted on mosquitoes as seen in Bargren and Nibley (1956), but Kohler and Fox (1951) ran a test in Puerto Rico which showed that Culicoides were more attracted to a light trap painted chrome yellow than to one painted forest green.

Habitats

Culicoides have been reported as utilizing a rather large variety of habitats, but all sites must be moist if not actually wet. They are found in both fresh and salt water situations as noted by Thomsen (1937) and Séguy (1950). A survey of the literature indicates they breed most abundantly in salt water tidal marshes. This type of habitat has been investigated by Williams (1956) in Bermuda, by Carpenter (1951) and Woke (1954) in the Panama Canal Zone, by Forattini et al. (1957) in Brazil, and by Sailer et al. (1956) in Alaska. Two rather thorough studies of this type of habitat were those of Dove et al. (1932) and Hill et al. (1934) who studied certain ecological niches within some southeastern Atlantic coastal marshes such as drainage ditches, open grassy areas, shaded areas, etc.

Inland saline areas also yield Culicoides, as summarized by Séguy (1950). Such an area exists at Saltville, Virginia, as reported by Snow et al. (1957) and Wirth and Jones (1957). Most of the reared material in our study came from this locality.

Fresh water breeding sites have been studied in England by Hill (1947), in Georgia by Williams (1955a), in Michigan by Williams (1955b), in Texas by Wirth and Bottimer (1956), and in Virginia by Wirth (1951) and Murray (1957).

Tree holes are the breeding sites of a number of Culicoides species. This type of habitat has been reported in Virginia by Pratt (1907) and more recently by Wirth and Jones (1956). Some of the other authors who have mentioned this habitat are Snow and Pickard

(1953) in the Tennessee Valley, Hinman (1932) in Louisiana, Fox and Kohler (1950) in Puerto Rico, and Foote and Pratt (1954) throughout the eastern United States.

Some other breeding sites are human sewage as studied by Jones (1959) and Pratt (1907), horse's hoof prints according to Downes (1950), cacti studied by Ryckman (1960), slime-covered bark according to Thomsen (1937), and rotting banana stems in Africa studied by Hopkins (1952). Wirth and Blanton (1959) list other references and breeding sites such as compost piles; rotting leaf mold; decaying fruit and stems of plants; mud or organic material in or at sides of holes in bamboo joints, coconut shells, rotten boat bottoms; wet debris in leaf or flower axils of pitcher plants, aroids, bromeliads; and crab holes. It can thus be seen that water is the common ingredient provided by all these situations.

Handling Specimens

The problem of developing an efficient method of removing larvae from their substrate has occupied the attention of several authors. Two basic types of techniques have evolved over the years. One is a sifting procedure using screens of decreasing mesh. Thomsen (1937), Hill (1947), Kettle and Lawson (1952), Beck (1952), Bidlingmayer (1957), and Becker (1958) used this technique.

The second basic technique is that of elutriation. The last four authors mentioned above followed the sifting procedure by

elutriation with magnesium sulfate. By this method the debris was placed in a concentrated solution of magnesium sulfate and the larvae were then floated out of the substrate. Elutriation using only water to flood the substrate was helpful to Dove *et al.* (1932), Hull *et al.* (1934), Smith and Lowe (1948), Carpenter (1951), Forattini (1957), and Jamnback and Wall (1958). Lattin in Usinger (1956) suggested a similar procedure using calcium chloride to flood the debris.

Counting these minute creatures is important in population surveys, but very time-consuming. Therefore, techniques for estimating larvae and adults have been employed by some researchers. Wirth and Bottimer (1956) counted the number of Culicoides in a 0.1 pint aliquot and estimated the total from that. Snow *et al.* (1957) after extracting moths and beetles, determined the number of Culicoides in 10cc. out of the first 100cc. of material, and thereafter 10 per cent of the total volume was sampled and the totals estimated. They counted 85,105 Culicoides in this way over a three year period.

Mounting

Because of their small size, most authors agree that Culicoides are best examined if they are mounted on microscope slides. A number of techniques are found in the literature such as those of Malloch (1915, 1916) using Canada balsam, Fox (1942, 1946) using creosote, Boesel and Vaughn (1951) using creosote and diaphane, Lee

and Reye (1952) using thin xylol-balsam, Ewen and Saunders (1958) and Saunders (1959) using creosote oil followed by Canada balsam, and Campbell and Pelham-Clinton (1960) who recommend a phenol-Euparal technique.

Our technique has been the one recommended by Wirth and Blanton (1959). It was also mentioned by Wirth (1952) and discussed by Arnaud (1956) who also listed a potassium hydroxide - balsam technique and a cellosolve-balsam mountant. The Wirth procedure involves clearing in phenol and mounting in 50-50 phenol-balsam mixture.

Identification

The earliest known reference to these minute flies was by Mouffet (1634) who referred to small blood-sucking gnats popularly known as "midges" in England. Derham (1713) was the first to give a description of their life-history as quoted in Edwards et al. (1939). They are included in the great work of Linnaeus (1758), but it was not until 1913 that Patton in India and Lutz in Brazil gave more precise descriptions of larvae and pupae. The family Ceratopogonidae was established as a subfamily of the Chironomidae by Meigen (1803) and separated to full familial status by Malloch (1916). The genus Culicoides was erected by Latreille (1809).

Some other good descriptions have been published by Winnertz (1852), Rieth (1915), Goetghebuer (1919), and Kieffer (1925) on some of the European species, and by Carter et al. (1920) of a number of

African species. Thienemann (1928) and Mayer (1934) have summarized the life-histories of the European species. Since that time the literature relating to all aspects of the genus Culicoides has been steadily increasing and we shall only deal with those references which contain keys to Culicoides, as these are the most valuable tool to those wishing only to identify to species.

A key to Australian species of Culicoides was published by Lee and Reye (1953), to Bermuda species by Williams (1956) and Wirth and Williams (1957), to British species by Edwards et al. (1939) and Campbell and Pelham-Clinton (1960), to French Culicoides by Kieffer (1925), to the species found in Japan, Korea and Ryukyu Islands by Arnaud (1956), to those of Micronesia by Tokunaga and Murachi (1959), to the Neotropical ones by Forattini (1957), to the Culicoides midges of Panama by Wirth and Blanton (1959), and to those of the Soviet Union by Gutsevich (1960).

The first taxonomic study in the United States was undertaken by Malloch (1915), although several species had been named previously by earlier entomologists. Hoffman (1925) reviewed all previously described species of Culicoides occurring in North and Central America and the West Indies and gave a key to females. A very good taxonomic study was published by Root and Hoffman (1937). It includes a key to almost all of the eastern species as well as some western and Mexican ones. Thomsen (1937) published a key to pupae of several Culicoides of the northeastern states.

Two excellent British papers, Edwards et al. (1939) and Hill (1947), include information on some species which also occur in North America.

A key to Culicoides females of the Caribbean region appears in an article by Fox (1946). In 1951, Wirth published a paper dealing with Culicoides in Alaska and included keys to females and male genitalia. Wirth (1952) presented a key to California Culicoides which is also found in Usinger (1956). Johannsen (1952) published a key to Culicoides in Connecticut. A key to Culicoides of the Americas was included in Fox's (1955) catalogue of the bloodsucking midges. This key was amended by Jones and Wirth (1958). Wisconsin Culicoides females were keyed by Jones (1956). The most frequently used keys are those of Foote and Pratt (1954), who have in their monograph keys to adult Culicoides females, to females based on structural characters, and to male terminalia.

Some other keys are also available to specific groups of species within the genus. For instance, Fox (1948) and Wirth and Blanton (1956) published keys to the subgenus Hoffmania. A key to the covagarciai group of Culicoides appears in Wirth and Blanton (1956) and to the unicolor complex in Wirth and Jones (1956). Wirth and Hubert (1959) have a key to the new subgenus Trithecoides and the same authors later (1960) published one on the copiosus group. Other such specialized keys may be found in the literature especially in Fox (1955).

A new concept, a key to the nearctic species of Culicoides based primarily upon the tibial comb of the hind tibia of both sexes, was published by Lewis (1956).

And finally, we must include the list of Culicoides species of the world recently (1959) published by Vargas and Garza.

Culicoides Abundance

Examples of great concentrations of Culicoides are found in the literature. Murray (1957) collected up to 45,600 Culicoides in a single trap in one night at Mt. Solon, Virginia. Some indications of estimated larval abundance in favorable habitats were given by Downes (1958) who found tundra species attaining populations of nearly one thousand to the square foot in soil around shallow pools in northern Canada, while Becker (1958) found as many as 1600 larvae in mud samples measuring 25 cubic inches taken from certain Scottish coastal salt marshes.

Virginia Culicoides

A survey of the literature reveals that the species listed below have been collected in Virginia. The initials refer to the following articles: FCP - Pratt (1907), WWW - Wirth (1951), F & P - Foote and Pratt (1954), W & J - Wirth and Jones (1956), WSM - Murray (1957), SPM - Snow et al. (1957), WWJ - Wirth and Jones (1957), J & W - Jones and Wirth (1958).

C. arboricola - Alexandria (F & P), Mt. Solon (WSM)

C. baueri - Mt. Solon (WSM)

C. biguttatus - Falls Church (F & P), Mt. Solon (F & P, WSM, WWW)

C. boringueni = hinmani - no locality given (J & W)

C. crepuscularis - Mt. Solon (F & P, WSM)

C. footei - Alexandria, Falls Church, Mt. Solon (WWW, W & J)

C. guttipennis - Alexandria (F & P), Bluemont and Woodstock (F & P, FCP), Mt. Solon (F & P, WWW, WSM), Paris, Skyland, Vienna, Warrenton (F & P)

C. haematopotus - Alexandria, Falls Church (F & P), Mt. Solon (WWW, F & P, WSM)

C. namus - Mt. Solon (WSM)

C. obsoletus - Alexandria (F & P), Mt. Solon (WWW, WSM, F & P) Skyland (F & P)

C. piliferus - Alexandria, Falls Church (F & P), Mt. Solon (WSM, F & P)

C. snowei - Falls Church, Alexandria (W & J)

C. spinosus - Falls Church (F & P), Mt. Solon (WSM)

C. stellifer - Falls Church (F & P), Fairfax Co. (FCP), Mt. Solon (WWW, WSM)

C. travisi - Alexandria, Falls Church (F & P), Mt. Solon (WWW, WSM)

C. variipennis - Richmond (FCP), Mt. Solon (WSM, F & P), Saltville (SPM, WWJ)

C. venustus - Falls Church, Mt. Vernon (F & P), Mt. Solon (WSM, F & P)

C. villosipennis - Mt. Solon (WWW, WSM, F & P)

Rearing

Laboratory rearing of Culicoides is a commonly employed method of studying these insects. A general account of rearing aquatic insects appears in Usinger (1956). Rearing larvae to obtain adults is a technique frequently used in survey work. Thus Fox (1949) did this in Puerto Rico; Woke (1954) reported rearing Culicoides in Central America; Coher et al. (1955) did this in New England; Snow et al. (1957) reared them from the Tennessee Valley; Wirth and Hubert (1960) utilized reared material obtained from Jones in Texas and Adachi in Arizona when they studied cactiphilic Culicoides; and Foote and Pratt (1954) reared many species while preparing their monograph on Culicoides of the eastern United States.

Hill (1947) reviewed the early accounts of laboratory rearing of Culicoides. The earliest attempt was made by Patel (1921) who induced engorged females to oviposit on moist blotting paper in tubes. Atchley and Hull (1936) recorded oviposition on moist blotting paper up to 12 days after a blood meal. Dove et al. (1932) were able to keep larvae alive up to 6½ months on material taken from their natural habitat. Steward (1933) was able to observe the complete development of Culicoides from egg to adult in cages covered with

fine wire gauze. A number of other workers have kept adults alive for as long as three weeks in a variety of cages: see Sharp (1928), Buckley (1934, 1938) and DuToit (1944).

The most successful rearing techniques seem to be those which attempt to duplicate the natural environment of the insect. This idea was used by Hill (1947), Hopkins (1952), Kettle and Lawson (1952), Woke (1954), Wirth and Bottimer (1956), Becker (1958), Ryckman (1960) and others.

Artificial conditions have been utilized more or less successfully by Hopkins and Nicholas (1952), Bidlingmayer (1957) and Jamnback and Wall (1958).

The most extensive and successful Culicoides rearing program ever undertaken was that developed by Jones (1957, 1960). His mass production techniques were carried out in a thermostatically controlled room and required one person's full-time attention. However, it produced an average of 1000 adult flies per day. The larval medium consisted of cow manure, black soil, yeast and water. The colony became adapted to laboratory conditions over a two-year period and had passed through 48 generations at the time the work was reported.

Longevity

It is apparently not known how long adult Culicoides remain alive in nature, but Williams (1955) postulated that C. haematopotus

may live 60 plus days as an adult and that C. stellifer may live 40 days at the particular time and place that he collected them in Georgia.

Several authors have remarked upon the life-span of the imago under laboratory conditions. Dove et al. (1932) found that C. canithorax and C. dovei live only ten days under laboratory conditions. Sharp (1928) kept C. austeni alive for two weeks in his African laboratory. In Malaya Buckley (1938) was able to keep C. pungens and others alive in the darkness of an incubator. C. nubeculosis survived more than a month under the conditions maintained by Downes (1950, 1958) in his laboratory in England. In Central America Woke (1954) had some Stilobezzia coquilletti still alive after 37 days. These were in fresh water taken from a shallow stream.

Synovitis Literature

Early Reports: Although practically nothing has been published on the natural vectors of infectious synovitis, the literature on other aspects of the disease has been increasing in recent years.

Infectious synovitis was first thought to be a form of bacterial arthritis by the early investigators of the disease. In 1892 Lucet described an "infectious osteo-arthritis" of young geese in France, indicating Staphylococcus aureus as the agent. Later Freese (1907) recognized a similar condition in geese and ducks in Germany and described acute and chronic forms, as did Loeffler (1910). In Holland,

Van Heelsbergen (1919) recorded the disease. Hutyra and Marek (1926) refer to it as "Staphylomycosis," and Ward and Gallagher (1920) as "Osteo Arthritis of Young Geese and Ducks." In 1929 Van Heelsbergen discussed other reports of the disease in geese and ducks in Germany and Holland. He concluded that it occurs less often in chickens than in geese and ducks. He cited Stroh as having reported the condition in grouse in Germany.

In 1933 Jungherr reviewed the literature on staphylococcal arthritis-like diseases in birds, and reported an outbreak in young turkeys in Connecticut. Reis and Nobrega (1935) described three cases of staphylococcal arthritis in chickens, two in pigeons, and one in a canary in Brazil. They produced arthritis in a chicken by inoculation directly into the tibiotarsal joint. Another culture, isolated from the pigeon, produced arthritis in other pigeons, but not in chickens when inoculated intravenously. They also reported considerable variation in the biochemical activities of the staphylococci isolated by them.

In 1940 Gwatkin reported an outbreak of staphylococcal infection in Barred Plymouth Rock males. Jungherr and Plastridge (1941) reported another outbreak in Connecticut, this time in five-month old chickens following the installation of peckguards through the nostrils which they thought to be a contributing factor in the spread of the disease. The principal lesion was swelling of the tibiometatarsal joints. Madsen (1942) gave a general description

of the disease and was the first to propose the name synovitis. In 1946 Van Ness reported arthritis and "keel cysts" in two month old chickens and isolated Staphylococcus citreus from them.

Rowlands and Smith (1945) reported acute cases of staphylococcosis of young geese in North Wales with a mortality rate as high as 25 per cent. Both leg and wing joints were swollen. They also suggested the possibility of "carrier" birds being introduced into the flock with contamination of nesting places or drinking pools being sources of infection. Hinshaw (1948) gave a general discussion of synovitis in turkeys. Moordsy (1948) reviewed the literature on avian staphylococcosis, and also discussed Bacterium arthropyogenes, Escherichia coli, and Salmonella pullorum in relation to avian arthritis.

A number of other bacterial organisms have been reported as the etiological agent of arthritis affecting poultry. These bacteria with the references to the articles where they are discussed have been listed by Wills (1954, 1955) and Wills and Delaplane (1955). They are Bacterium arthropyogenes (Nobrega, 1940), Escherichia venezuelensis (Gallo, 1942), Salmonella pullorum (Beaudette, 1936), Pasturella multocida (Thorp et al., 1931), and Erysipelothrix rhusiopathiae (Beaudette and Hudson, 1936; and Graham et al., 1939). Hinshaw and McNeil (1952) were able to transmit synovitis only by intravenous inoculation using certain strains of Micrococcus pyogenes var. aureus. They also refer to a possible relationship between

mosquito decrease and incidence of the disease. Fahey (1954) found this same organism and others in an outbreak in turkey poults. Some other accounts of the disease are those of Olson et al. (1954) in West Virginia, and Coffin (1955) who reported this disease in the eastern United States. Jungherr (1954) reported the disease in Connecticut and Dunlop (1954) reported it in New Hampshire.

Virus-related Studies: A new trend in the study of synovitis was begun by Wills (1954a, 1954b, 1955) who indicated that a large particle virus, a pleuropneumonia-like organism (PPLO), or a small unknown bacterium is the cause of infectious synovitis in chickens. Markham (1954) in a personal communication to Wills and Delaplane (1955) reported pleuropneumonia-like organisms isolated from chickens infected with arthritis. Other studies on the agent causing infectious synovitis have been published by Cover (1954) and Olson (1954). Snoeyenbos and Olesiuk (1955) studied the agent in turkeys, and while studying the etiology of the disease Cover (1956) reported finding a small bacterium or large particle virus.

A PPLO produced arthritis in chickens during researches by Wasserman et al. (1953) and Olson et al. (1955). It was established by Olson et al. (1956a, 1956b) that a large particle virus caused the disease. Other studies on the agent and etiology of the disease were published by Leece and Sperling (1955), Leece et al. (1955), Cover and Galeta (1955) and Cover et al. (1956).

Differences between viral infectious synovitis and PPLO synovitis were noted by Olson et al. (1957), but the two types could not be distinguished by blood counts or hemoglobin values. Cover and Benton (1957) found the viral agent distributed in almost all body tissues. By their methods a true viremia was produced 48 hours after innoculation and persisted until the 10th day after inoculation.

Studies related to the effects of the organism on the host have been published by Olson (1954) and Olson and Munro (1954) who dealt with the haematology and histopathology of the disease. Olson (1955) gave a general account of infectious synovitis up to that time. Sevoian et al. (1958) also presented a general discussion of the disease, especially from the histopathological point of view.

Benton and Cover (1959) demonstrated that the agent is present in infectious levels in the blood by the eighth hour after intravenous inoculation, and after intramuscular inoculation by the 48th hour. They found the agent persists in the blood until at least the 15th day after inoculation. Wichmann et al. (1960) reported a method of propagating the infectious synovitis agent using Simm-Sanders medium containing fragments of chicken embryo tissue. Chalquest and Fabricant (1960a, 1960b) have conducted some studies which indicate a PPLO is the agent.

The question of egg transmission has been remarked upon and sometimes demonstrated by Olson et al. (1954), Wills (1954), and Thayer et al. (1958). Snoeyenbos and Basch (1958) reported findings

which supported the observations of those previous workers, that is: transmission through eggs may occur from hens exposed while young to the agent of infectious synovitis.

Scott (1950) has reported that failure in creatinine retention may result in swelling of the tibiometatarsal joint, one of the symptoms of infectious synovitis.

IV. PROCEDURE AND TECHNIQUES

Selection of Study Areas

Most of the survey and transmission work in this study was done in southwestern Virginia with Blacksburg as the center of operations. However, occasional trips were made to Elkton and Harrisonburg in the poultry-raising areas of the upper Shenandoah Valley in Rockingham County.

In 1959 most of our survey work was done at Ferrum (Franklin County) on the farm of Mrs. Frances Buckner because her flock of Rhode Island Reds was approximately 60 per cent infected with synovitis. The disease had been diagnosed by Dr. W. B. Gross, Department of Veterinary Science, V. P. I. The presence of the disease made this an ideal place for trapping engorged Culicoides females for transmission studies.

In 1960 most of the survey work was done in Blacksburg. Traps were easily maintained in a woods on the V. P. I. farm and in outdoor chicken and turkey shelters.

The saltmarsh at Saltville, Virginia, in Smyth County served as our source of supply for Culicoides variipennis australis Wirth and Jones (1957) larvae used in the rearing, longevity, and transmission studies undertaken in 1960.

Trapping was occasionally done in other localities that seemed suitable for our purposes. Therefore, some trapping was done on a chicken farm near Poplar Camp in Wythe County, on a chicken farm near

Bent Mountain in Roanoke County, and in wooded mountains around streams near Newport but in Montgomery County.

Trips were also made to the Broad Run Wildlife Management Area near New Castle in Craig County to collect Culicoides larvae for rearing purposes.

Trapping

Description of the Traps: The traps used were standard New Jersey light traps as described by Usinger (1956). Both electric- and battery-operated traps were used.

In order to achieve some live trapping in 1959 at Ferrum and Poplar Camp, three of the traps were fitted with a round pasteboard box in place of the usual alcohol or cyanide collecting jar. These boxes were of the type used for shipping glass gallon jugs of insecticides. A circular hole approximately four inches in diameter was cut in the lid so it would fit snugly onto the screen funnel inside the traps. The diameter (8-1/2 inches) of the box was just a little less than the inside diameter of the trap, so it fit conveniently in place. A circular hole approximately six inches in diameter was cut in the bottom of the box and covered with a fine copper screen on the inside. This permitted air to pass through the box, yet prevented the insects from escaping. The box was held in place by a loop of strong binding twine which permitted easy removal. As soon as the

box was slipped out of the trap (with the fan still turning) the top hole was covered with a round piece of cardboard which was held in place by a piece of cheesecloth and two large rubber bands. Most of the insects survived the 60 mile trip back to the laboratory where the Culicoides could be sorted out.

A larger trap utilizing a circular ultra-violet lamp was also employed at Ferrum, but it offered no advantages over the New Jersey traps, and its use was discontinued.

Other than the live trapping mentioned above, all insects were collected directly into 70 per cent ethyl alcohol in pint jars screwed onto the receptacle at the base of the funnel in the traps.

Location of Traps: The traps were hung by a rope attached to a rafter in the poultry house or from a limb of a small tree in the woods. They were all placed so that the light bulb was approximately three to six feet above the floor or ground.

In the large poultry houses at Elkton and Poplar Camp a trap was placed at each end of the house. A room between them thus confined the light to separate ends of the building. In all the other poultry houses, there was just one trap per room.

At Ferrum, Elkton, and Poplar Camp the two buildings which housed the poultry were so placed that one was nearer to a woods than the other. It was thought that this might make a difference in the total numbers of insects captured in each building because of the proximity of one of the buildings to a potential breeding site.

Battery operated traps were also set up outside the poultry house on the side away from the woods at Poplar Camp and in a nearby woods at Poplar Camp and Elkton.

At Blacksburg a battery-powered trap was hung in the woods beside the Price's Fork Road. Another trap was placed in an outdoor poultry coop next to the Veterinary Science Laboratory about three-fourths of a mile away from the woods trap and on slightly higher ground.

The Bent Mountain traps were placed inside poultry houses. The Newport trap was located beside a small spring-fed stream on the side of Sinking Creek mountain at approximately 2000 feet elevation one-half mile off route 621 and about two miles from route 460.

The traps were operated all night in every case. The Blacksburg traps averaged 14 hours per night. A thermo-humidigraph was used to record temperature and relative humidity at this latter location. In all instances the dates used are the dates the traps were started.

Handling Captured Insects

The insects that were live-trapped in 1959 were brought back to the laboratory in the circular boxes. A hose connected to a tank of carbon dioxide was inserted through a hole in the piece of cardboard covering the opening in the lid. This hole was stoppered with a cork at other times. A stream of gaseous carbon dioxide was allowed to

enter the box for approximately one minute. Then the anaesthetized insects were all dumped into a white enamel pan. The Ceratopogonids were quickly removed with an aspirator. The others were placed in 70 per cent alcohol after being killed in a cyanide jar.

The Ceratopogonids were given some more CO_2 and quickly sorted under a dissecting microscope and the engorged Culicoides females removed to holding cages. The remainder were killed in a cyanide jar and transferred to alcohol.

The insects captured by the ordinary light traps were forced directly into jars of 70 per cent alcohol. These jars were labelled, capped and brought into the laboratory where the contents were transferred to pint Mason jars for storage until the insects in them could be sorted.

Sorting

All of the insects preserved in alcohol were sorted into definite categories: into orders for all except the Diptera which were separated into Brachycera and into each family of Nematocera. The family Ceratopogonidae was further sorted and the genus Culicoides removed. Finally this genus was separated into species. After being sorted, the specimens were labeled and stored in 70 per cent alcohol in four dram vials stoppered with number 0 Neoprene corks. A color code by year was set up: 1958 collections - gray corks, 1959 collections - black corks, 1960 collections - green corks.

Slide Mounting

Culicoides species were mounted on slides following a technique published in Wirth and Blanton (1959). The description of this method is copied here in its entirety.

INSTRUCTIONS FOR PREPARING SLIDES OF

CERATOPOGONIDAE AND CHIRONOMIDAE

W. W. Wirth

March, 1959

1. Material preferably collected and stored in 70 per cent alcohol but dry specimens will also work with this method.
2. Stock solutions and materials to be prepared.
   a. Pure phenol (carbolic acid) crystals diluted with enough absolute (100 per cent) ethyl alcohol to prepare a saturated solution (with a layer of about half by volume of phenol crystals in the bottom of the bottle with the phenol-alcohol solution above). The latter is poured off and used. Can be prepared any time in advance.
   b. A bottle of balsam-phenol mixture, prepared by placing half by volume of the above liquified phenol and half of ordinary Canada balsam of consistency used for making slides or a little thicker. This mixture must be prepared at least a day in advance of use and no more than a month ahead, as it turns dark with age.
   c. Ordinary Canada balsam for replenishing the phenol balsam mixture in the slides as they are dried in the oven.

3. Procedure

a. Put enough liquid phenol in a stender dish to cover specimens. Transfer specimens from alcohol or dry into the mixture and allow to relax and clear for about 12-24 hours. Specimens may be left in the phenol indefinitely. As many specimens as necessary, up to about 10 per cc. of phenol may be placed in each dish.

b. Transfer the specimens to a dish of the phenol-balsam mixture from which they may be mounted on slides at once, but do not leave them in the mixture longer than an hour or two before dissection as they tend to harden somewhat in the mixture.

c. With pipette or forceps, transfer a specimen with a large drop of the phenol-balsam mixture to a slide for dissection. For females cut off the head and orient face side uppermost, cut off one wing and the abdomen and orient the latter ventral side uppermost. Place all including thorax and legs under one coverslip or put the wing under a separate one if desired. Add enough phenol-balsam mixture to fill the space under the coverslip, label slide and put in a drying oven to dry under warm heat only, for one to two weeks, until the slide has hardened, at least around the edges so that the slip does not slip under moderate pressure sideways.

For males, cut off one wing, the head and the genitalia. Cut the abdomen just ahead of the genital segments so the latter can be oriented ventral side uppermost. Replace the mixture which evaporates with balsam every day or two until no further evaporation takes place. 16mm. circular coverslips are the most satisfactory. Put one specimen to a slide. Shrinkage of antennae, spermathecae, etc. may be avoided by rinsing the specimens in alcohol to keep water out of the phenol. Sometimes dilution of the phenol-balsam mixture with more liquid phenol will stop shrinkage.

Larval Collections

Larvae were collected primarily from two types of habitats - rot holes in stumps (tree holes) and soft mud. On July 22, 1959 Culicoides larvae were taken from a hole in a stump beside the road in the Broad Run Wildlife Management Area. The larvae with some water and debris from the hole were transported back to the laboratory and poured into 7-1/2 x 12 x 1-3/4 inch enameled trays. The trays were raised at one end so that the water depth varied. They were covered with a piece of glass to prevent evaporation.

On July 31, 1959 Culicoides larvae were collected from tree holes in the woods on the V. P. I. farm. They were placed in enameled pans in the laboratory as above. Again on August 26, 1959 larvae

were collected from two tree holes in the V. P. I. woods. Sixty larvae from a small stump and 22 larvae from a large stump were placed in trays in a controlled temperature cage put at our disposal by the Department of Veterinary Science, V. P. I. Sixty larvae from each stump were placed in an air-conditioned room in the laboratory. The temperature at time of collection was 78° F., in the cage 76° F., and in the room 67° F.

The 1960 larval collections were as follows:

April 1, 1960 - 214 larvae were collected from the large tree hole in the V. P. I. woods. They were placed in pan #5 in an incubator kept at 24° C.

April 25, 1960 - 63 larvae were collected from the same site when the water temperature was 60° F. and the air temperature 88° F. with the sky cloudy. They were put in pan #6 and #7 in Room 103, Price Hall.

April 28, 1960 - 250 larvae were siphoned from small tree holes in three different stumps on Brush Mt. near the Montgomery-Giles County line at about 2100 feet elevation. Air temperature was 76° F. and water temperature 58° F. The day was sunny. Most of the larvae were established in pan #8. (Five were put in pan #9 with tap water and dog food pellets to test an artificial medium. Within two days a bacterial scum had formed and on the fourth day the contents had to be discarded.) These and all subsequent pans were maintained at room temperatures in Room 103, Price Hall.

June 14, 1960 - Leaf litter and a little water was collected from the same stump visited on July 22, 1959 in the Broad Run Area. This was established as pan #9. Mud was collected from the edge of two different ponds on this trip and placed in pans #10 and 11.

June 15, 1960 - Muddy water was collected from cattle hoffprints behind a shed beside U. S. 11 not far from Rural Retreat in Wythe County. These were placed in pan #12.

June 15, 1960 - Saline water, algae, Culicoides larvae and pupae were collected in a stagnant pond in the saltmarsh at Saltville, Virginia. The pond was surrounded by Juncus gerardi and contained a heavy agal growth which was probably Lyngby aesturii (Mert.) Lieb., Anacystis dimidiata (Kuetz.) Drou. and Daily, and A. thermalis (Menegh.) Drou. and Daily, common species known from fresh and brackish water. The algae were named by Snow et al. (1957) who collected at the same place. They found a sample of the water contained 26.2 grams per liter of NaCl. Larvae and pupae of the saltmarsh mosquito Aedes sollicitans (Walk.) also were taken from the pond. All of these larvae were established in pans #13, #14, #15, #16, and one earthenware crock numbered 47.

June 28, 1960 - At the Saltville marsh larvae were collected from mud at the edge of the large pool in the center of the marsh. The depth of the water over this mud varied from zero to four inches. These larvae were placed in about a dozen crocks with mud and water. The crocks were covered and upon return to the laboratory

an emergence cage was placed on the crock as described below. On July 5, 1960, the contents of two of the crocks were poured into pans #17 and #18.

July 6, 1960 - Mud and water were collected in a spring runoff in a pasture on Slusser's Chapel Road in Montgomery County. These were put in pan #19.

July 7, 1960 - Water containing unidentified pupae and debris was collected in a runoff from the V. P. I. dairy and swine barns. It was placed in pan #20.

July 15, 1960 - Mud and water were collected in a spring runoff at Nature Camp, Vesuvius, Virginia. This was established as pan #21.

July 29, 1960 - Water was collected with one Culicoides larva and one pupa from a stump in Poverty Hollow, six miles from Blacksburg, in Montgomery County. This was put in pan #22.

August 5, 1960 - Water was collected from tree holes on Brush Mt. This was the same site as that used on April 28, 1960. No larvae were seen, but water was placed in pan #23.

August 17, 1960 - Culicoides larvae were collected from four sites in the Saltville marsh as follows:

Site #1: Small pond approximately three feet by six feet covered with an algal mat. This was the same pond as the one used on June 15th trip. About 40 larvae per dipper of water were dislodged from the underside of the algal mat. More than 200 larvae of all sizes were collected at about 11 A.M.

Site #2: Flooded area of the rush Juncus gerardi. The larvae were collected at the rate of about 15-20 per dipper. More than 300 larvae were brought back from this site, which was on the edge of the mud flat which produced so many larvae on June 28. These were collected at about 10 A.M.

Site #3: The same area as used on June 28, but on this trip it was covered by one or two inches of water. Collecting consisted of scooping up one or two dippers of mud from the top inch or more and placing it in a gallon crock. Four crocks of mud and one pint jar were thus filled. The number of larvae was not known. This was done about noon on a very hot, sunny day.

Site #4: A three by five feet pool covered with algae behind a brick pump house in a different part of the marsh than above. This site yielded 50-100 larvae of all sizes per dipper. Four crocks were filled with water and algae about 2 P.M.

Upon returning to the laboratory, all of these crocks were propped up at an angle and an emergence cage placed over each of them.

September 7, 1960 - Larvae were collected from a 1-1/2 x 2-1/2 feet pool completely devoid of vegetation in or near it. Collecting was done about 6 P.M. Upon arriving at the laboratory, these larvae were put in pan #24.

September 8, 1960 - Collecting was done on the mud flat (Site #3 above) at Saltville. The top one to two inches of very wet, black

mud was scraped off and placed in a crock so that each crock held about 500 cc. of mud plus one-half inch of water. This was done about 10 A.M. Thirty-three filled crocks were brought back to the laboratory and set up as previously.

October 15, 1960 - Four crocks of mud containing 500 cc. each were collected from the same site as before on the mud flats. This was done about 2 P.M.

November 13, 1960 - Four crocks of mud were collected from the Saltville mud flats as follows:

1. Top 1/2 inch to 1 inch of mud covered by 1/4 inch to 1/2 inch of water at about 11:30 A.M.
2. Second 1 to 2 inches of mud from same place as #1.
3. Top 1 to 3 inches of mud not covered by water. Nearest surface water one foot away.
4. Top 1/2 inch of mud covered by 1/4 inch or less of water. The time for this collection was about noon.

During the month of November the mud from six crocks collected on September 8 was gone through carafully and all the larvae still present were counted.

Rearing

As adults emerged from the substrate in the enameled trays, they were removed once or twice a day with an aspirator and transferred

to holding cages. However, a few sometimes escaped while being aspirated out of the pan, so it was decided to use the earthenware crocks as rearing vessels with emergence cages on top.

The holding cages and emergence cages were constructed from round ice cream cartons. Both pint- and gallon-sized cartons were utilized. A circular hole 1-7/8 inches in diameter was cut in the bottom of the pints and a circular hole 2-3/8 inches in diameter was cut in the bottom of the gallon-sized cartons. These holes accommodated the bottom part of round pill boxes. The top of the cartons were pushed out and saved to be used as crock covers. They were placed on the carton by pieces of clear polyethylene plastic held in place by the remaining ring of the carton top. Two holes were cut in this plastic. The smaller was covered with a piece of silk bolting cloth glued in place. The larger was closed by a cork.

When the cartons were used as a holding cage, several layers of paper toweling cut to size were put in the pill box which was then filled with water. To provide nourishment for the adult Culicoides, one or two raisins were dropped into the cage. It was not necessary to split the raisins and by not doing so, fewer insects stuck to them.

The cages containing the adults were kept inside a dark incubator at 75° F. and approximately 40 per cent relative humidity as recommended by Jones (1957, 1960). They were checked daily and any dead Culicoides were removed and preserved in four-dram vials of

70 per cent alcohol and labeled. Many were subsequently mounted on slides for further study.

When the cartons were used as emergence cages, the pill boxes were removed and the cartons were placed over the crocks of mud containing larvae. Holes the same size as those left by the removal of the pill boxes from the cartons had been made in the covers of the crocks. Then, as the adults emerged from the mud, they flew towards the light coming through the holes and up into the cartons above. From there they were easily removed with an aspirator and transferred to numbered holding cages.

Longevity Study

An adjunct of this rearing work was the longevity study. It soon became apparent that under the conditions maintained in the incubator, the adults were surviving for a rather long time. A great deal of mortality, however, occurred when they adhered to the water in the pill boxes. By covering the surface of the water with a fitted paper towel, this danger was reduced. Some water was necessary for survival, however.

Infectious Synovitis Study

Transmission Experiments, 1959: In 1959 attempts were made to transmit infectious synovitis to healthy chickens using wild-caught,

engorged Culicoides females.

On July 16, 1959, live-trapped insects were brought to the laboratory from the Buckner farm at Ferrum, Virginia. They had been trapped the preceding night in the chicken houses containing infected birds. It should be noted that these birds had been receiving aureomycin in their feed for approximately 17 days. Twenty Culicoides females were used. There were 14 C. arboricola, three C. ousairani, one C. guttipennis, and two others unidentified.

They were macerated in a tissue grinder with four ml. of sterile tryptose broth. This suspension was inoculated intravenously into two male, nine-week old, White Leghorn chickens. Each received two ml. of inoculum. The chickens were then placed in a cage and observed for signs of synovitis infection until August 26.

On July 24, 1959, a similar experiment was conducted. This time 40 live-trapped, engorged Culicoides females from Ferrum were macerated in five ml. of sterile tryptose broth. Two male and two female five-week old chickens were inoculated intravenously. No attempt was made to identify these punkies as to species. The chickens were observed periodically until August 26. The Buckner chickens had now been under aureomycin treatment about 25 days. There were no further tests in 1959 for this reason.

Transmission Experiments, 1960: In the summer of 1960 a series of tests was conducted in an attempt to demonstrate the viability of the infectious synovitis agent in a potential vector. The Culicoides

used were reared from larvae collected at Saltville, Virginia. All were identified as Culicoides variipennis.

The chickens used were White Leghorns raised in a disease-free environment in the laboratory. The age of the chickens used in the preliminary experiment was eight weeks as the tests began. Those used in the principal tests were four and one half weeks old as the tests began. Both males and females were used. The ages of the adult Culicoides were two to ten days.

The chickens used for feeding the insects were pre-inoculated with splenic suspensions containing the synovitis agent. Those in the preliminary tests each received 0.2 cc. on July 25, 1960. Those used in the principal tests each received 0.5 cc. on September 12, 1960.

After eight preliminary experiments it was found that the Culicoides variipennis females were most active and hence fed more readily under a bright flourescent light. In the first attempts to induce Culicoides to feed on the infected chickens a clear plastic cylinder one inch in diameter and three and one half inches long was used. Silk bolting cloth was fastened over one end. The other end was closed with a cork. The insects were aspirated directly into the tube. Then they were anesthetized with CO_2, and the tube was slipped over one leg of the chicken and plugged with cotton to prevent the insects from crawling out.

Another arrangement which we used for feeding Culicoides was devised by Scanlon (personal communication) for his studies on the

relationship of Culicoides to encephalitis. The plastic tubes mentioned above were cemented to a rectangular padded base. The insects were introduced in to the tube as before and the open end was placed over a patch of the chicken's skin which had been plucked clean. It was held in place by string or adhesive tape.

The final method was to slip one of the pint-sized cages over the chicken's head, stuff some terry cloth between the bird's neck and the hole and then introduce the insects. A cone fashioned from 1/4 inch hardware cloth was later added to prevent the chicken from shaking its head when the insects attacked it. The cage was held in place with adhesive tape.

During the feeding the chickens were always held in place in a restrainer consisting of a board and hooks to which two 1/4 inch chains and springs were attached. The chains passed over the bird's body with the springs so arranged that they were held under tension. Another spring at one end held their feet.

Various numbers of female Culicoides were induced to feed upon the pre-inoculated chickens at intervals of three, five, and seven days after inoculation. After being fed, the insects were kept in holding cages for intervals of one to six days.

The insects were then immobilized by freezing, and macerated in a tissue-grinder with one cc. of a 1:2 suspension of sterile tryptose broth. The clean chickens were inoculated intramusculary in the thigh with the suspension and then placed under observation for

approximately one month. These tests are summarized in Tables I and II.

As a part of the preliminary test, two chickens were each inoculated with 0.3 cc. of blood drawn from those chickens inoculated on July 25, 1960. These check birds were sacrificed on August 11, 1960, and examined for symptoms of synovitis. The inoculated chickens used in the preliminary test were killed and examined for synovitis symptoms on August 2. The pre-inoculated birds in the final test were sacrificed and autopsied on September 27.

The tests were terminated September 6 and October 19 respectively.

TABLE I

Preliminary Test of the Viability of the Infectious Synovitis Agent in a Potential Vector, C. variipennis.

Culicoides Date Fed	No.	Date Macerated	No.	Amount of Inoculum
7/28/60	20	7/29/60	20	0.95 cc.
7/30/60	51	8/1/60	27	0.70 cc.
		8/2/60	21	0.80 cc.
8/1/60	12	8/2/60	12	0.50 cc.

TABLE II

Test of the Viability of the Infectious Synovitis Agent in a Potential Vector, C. variipennis.

Culicoides Date Fed	No.	Date Macerated	No.	Chickens Inoculated	Amount of Inoculum
9/15/60	92	9/16/60	30	1	0.65 cc.
9/15/60	92	9/19/60	28	2	no data
9/17/60	410*	9/19/60	55	2	0.40, 0.35
9/17/60	410*	9/20/60	56	2	0.40, 0.20
9/17/60	410*	9/21/60	55	2	0.50 cc. ea.
9/17/60	410*	9/22/60	55	2	0.50 cc. ea.
9/17/60	410*	9/23/60	45	2	0.50 cc. ea.
9/19/60	181**	9/20/60	45	2	0.41, 0.35
9/19/60	181**	9/21/60	45	2	0.50, 0.64
9/19/60	181**	9/22/60	46	2	0.40 cc. ea.

* 114 and 135 "older" Culicoides fed on two chickens respectively. 161 Culicoides which had emerged the previous three days fed on another chicken.

** 52 "older" Culicoides fed on one chicken, whereas 129 Culicoides which had emerged two days previously fed on another chicken.

V. RESULTS OF INVESTIGATION

Insect Survey

Trapping in 1959: In the summer of 1959 light traps were operated for the most part in poultry houses to capture Culicoides specimens. These traps collected several species of Culicoides as enumerated below: Ferrum, Virginia (four traps in four separate rooms of two chicken coops)

Culicoides	July				August			
Species	2	8	15	31	6	13	20	Total
arboricola	2	7	20	48	64	15	20	176
crepuscularis	1	6	17	12	30	16	29	111
debilipalpis	-	-	3	-	-	-	-	3
guttipennis	6	14	7	26	36	40	25	154
haematopotus	1	-	-	1	2	1	3	8
obsoletus	12	-	8	587	454	68	96	1225
ousairani	-	-	-	-	-	-	11	11
stellifer	1	-	7	18	11	4	5	46
travisi	3	-	-	-	2	-	-	5
variipennis	-	-	-	1	-	1	-	2
venustus	-	3	-	6	16	3	1	29
villosipennis	1	3	15	35	8	10	31	103

At Poplar Camp, Virginia, traps were operated on three consecutive nights. Four traps were each placed in separate rooms of two

large chicken houses. One battery-operated trap was placed in the adjacent woods. The results follow:

Culicoides		Chicken Houses			Woods			
Species	August	3	4	5	3	4	5	Total
arboricola		9	2	11	-	-	-	22
biguttatus		1	-	-	-	-	-	1
crepuscularis		1	-	-	-	-	-	1
guttipennis		8	13	-	-	3	-	24
haematopotus		1	-	-	-	-	-	1
obsoletus		70	5	15	-	42	-	132
ousairani		-	1	-	-	-	-	1
piliferus group		1	1	-	-	1	-	3
stellifer		2	-	-	-	-	-	2
travisi		1	1	-	-	-	-	2
venustus		-	1	-	-	-	-	1
villosipennis		2	-	-	-	-	-	2
		85	22	15	-	46	-	168

Three traps were operated in two large chicken houses at Bent Mountain, Virginia, on the night of July 22, 1959, with the following results: C. crepuscularis - 1, C. obsoletus - 7, and C. venustus - 2.

Finally, mention must be made at this point of a very interesting specimen taken at Newport, Virginia, on July 17, 1959. While investigating a small spring-fed stream on a wooded mountainside the author

felt a Culicoides biting his hand. A vial was placed over the specimen to capture it. Dr. Wirth of the U. S. National Museum later identified it as C. paraensis, the only example of this diurnal, tropical species taken during this study.

Trapping in 1960: A battery powered trap was operated about three nights a week from April to September in the V. P. I. woods at Blacksburg. A trap in the poultry coop was operated on the same nights as the one in the woods, but from June to September. The traps were run continuously from about six in the evening until about nine the following morning. A summary of this trapping is found in Tables III-VII.

The trapping at Elkton, Virginia, on August 8 revealed the following species and the numbers of each taken.

Culicoides -Species	Upper Left Turkey Hse.	Upper Right Turkey Hse.	Lower Turkey Hse.	Woods	Total
arboricola	34	32	52	-	118
crepuscularis	9	8	2	-	19
guttipennis	32	13	13	3	61
obsoletus	155	231	75	-	461
ousairami	3	1	-	2	6
venustus	1	-	-	-	1
villosipennis	-	-	-	1	1
	234	285	142	6	667

The trapping at Newport and Saltville in August yielded scanty results as seen below:

C. stellifer	Newport	August 4	1
	Saltville	August 16	1
C. variipennis	Saltville	August 16	1

Effect of Trap Location: In order to better see how the location of the traps influenced the taking of Culicoides species we may regroup the results of the Poplar Camp (1959) and Blacksburg (1960) trapping in Table VIII. It should he noted that there were four electrically powered traps in the poultry houses at Poplar Camp and only one battery powered trap.

At Elkton the room nearest the woods and hence a potential Culicoides breeding ground was the one designated "Upper Left". We may disregard the results of the woods trap because it was just one single trap compared to the four in the turkey houses. A recapitulation of the totals reveals the following results of all species captured:

Upper Left - 234 Upper Right - 285 Lower - 142

It should be noted that all species except obsoletus were taken in greater numbers in "Upper Left" than in "Upper Right".

TABLE III

Culicoides Trapped in Blacksburg Woods, April and May, 1960.

Culicoides Species	April				May							
	2	9	18	25	5	6	24	25	26	30	31	Total
baueri	-	-	-	-	-	-	1	-	1	-	-	2
biguttatus	-	-	1	-	2	-	59	-	48	2	-	112
crepuscularis	-	-	-	-	-	-	1	-	-	-	-	1
haematopotus	-	2	7	1	-	1	-	-	1	-	-	12
obsoletus	-	-	5	1	-	-	2	-	1	-	-	9
piliferus group	-	-	-	-	-	-	3	-	2	1	-	6
spinosus	-	-	-	-	-	-	9	1	4	-	-	14
stellifer	1	1	-	-	-	-	-	-	-	-	-	2
travisi	-	-	2	1	-	-	80	-	41	-	2	126
variipennis	-	-	2	-	-	-	-	1	-	-	-	3
venustus	-	-	-	-	-	-	15	1	10	-	-	26
Totals	1	3	17	3	2	1	170	3	108	3	2	313

TABLE IV

Culicoides Trapped in Blacksburg Woods and Poultry Coop, June, 1960.

Culicoides Species	June 1		2		8		17		20		22	
	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC
baueri	-	-	-	-	-	-	-	-	-	-	10	-
biguttatus	2	-	9	-	-	-	1	-	-	-	12	-
crepuscularis	-	-	-	-	-	-	-	-	-	7	-	-
chiopterus	-	-	-	-	-	-	-	-	-	-	2	-
debilipalpis	-	-	-	-	-	-	-	-	-	-	2	-
haematopotus	1	-	1	-	-	-	-	-	-	3	4	-
obsoletus	1	-	-	-	1	-	-	-	-	8	33	-
ousairani	-	-	-	-	-	-	-	-	-	1	-	-
piliferus group	-	-	-	-	-	-	-	-	-	-	4	-
snowi	-	-	-	-	-	-	-	-	-	-	-	-
spinosus	-	-	8	-	2	-	2	-	-	-	2	-
stellifer	1	-	-	-	-	-	-	-	-	6	89	-
travisi	2	-	20	-	2	-	1	-	1	-	22	-
variipennis	-	-	-	-	-	-	-	-	-	-	-	-
venustus	-	-	-	-	-	-	-	-	-	3	9	-
villosipennis	-	-	-	-	-	-	-	-	-	-	15	-
	7	-	38	-	5	-	4	-	1	28	204	-

TABLE IV (Continued)

Culicoides Trapped in Blacksburg Woods

and Poultry Coop, June 1960

Culicoides June	23		24		27		29		30	
Species	W	PC	W	PC	W	PC	W	PC	W	PC
baueri	1	-	1	-	-	-	47	-	-	-
biguttatus	10	-	36	-	-	-	93	-	-	-
crepuscularis	-	-	-	4	-	2	2	-	-	1
ehiopterus	-	-	1	-	-	-	-	-	-	-
debilipalpis	-	-	-	-	-	-	-	-	-	-
haematopotus	1	-	3	-	-	-	6	-	-	-
obsoletus	3	-	28	-	-	1	14	-	-	-
ousairani	-	-	-	-	-	-	-	-	-	-
piliferus group	-	-	5	-	-	-	5	-	-	-
snowi	-	-	-	-	-	-	1	-	-	-
spinosus	12	-	1	-	-	-	11	-	-	-
stellifer	-	-	57	12	-	-	18	4	-	-
travisi	17	-	58	-	-	-	54	-	2	-
variipennis	-	-	-	1	-	-	-	-	-	-
venustus	-	-	1	1	-	-	1	-	-	-
villosipennis	-	-	-	-	-	-	3	-	-	-
	44	-	194	18	-	3	255	4	2	1

Totals: Woods 754

Poultry Coop 54

TABLE V

Culicoides Trapped in Blacksburg Woods and Poultry Coop, July, 1960.

Culicoides Species	1		2		3		6		7		8		17		20	
	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC
arboricola	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-
baueri	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
biguttatus	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
crepuscularis	-	-	-	-	-	-	-	17	-	2	-	23	-	-	-	-
guttipennis	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-
haematopotus	1	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
obsoletus	9	-	-	-	-	-	-	7	-	2	-	6	-	-	13	-
ousairani	1	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
spinosus	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
stellifer	5	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-
travisi	9	-	-	-	-	-	2	-	1	-	1	-	-	-	-	-
variipennis	1	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
vemustus	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	-
villosipennis	2	-	-	-	-	-	-	1	-	-	-	1	-	-	-	-
	48	-	-	-	3	-	2	27	1	5	1	34	-	-	17	-

TABLE V (Continued)

Culicoides Trapped in Blacksburg Woods

and Poultry Coop, July, 1960.

Culicoides Species	21		22		23		24		26		27		29	
	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC
arboricola	-	-	-	-	-	-	-	-	-	-	-	-	-	-
baueri	-	-	-	-	-	-	-	-	-	-	-	-	-	1
biguttatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
crepuscularis	-	4	-	-	-	-	-	-	-	-	-	-	-	-
guttipennis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
haematopotus	-	-	-	-	-	-	-	-	1	-	-	-	-	-
obsoletus	-	2	-	-	1	-	-	-	7	-	-	-	-	10
ousairani	-	1	-	-	-	-	-	-	-	-	-	-	-	-
spinosus	-	-	-	-	-	-	-	-	-	-	-	-	-	-
stellifer	-	-	-	-	-	-	-	-	1	-	-	-	-	-
travisi	1	-	-	-	-	-	-	-	-	-	-	-	-	-
variipennis	-	-	-	-	-	-	-	-	-	-	-	-	-	2
vemustus	-	1	-	-	-	-	-	-	-	-	-	-	-	-
villosipennis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1	8	-	-	1	-	-	-	9	-	-	-	-	13

Totals: Woods 83

Poultry Coop 87

TABLE VI

Culicoides Trapped in Blacksburg Woods and Poultry Coop, August, 1960.

Culicoides Species	1		4		5		8		11		17		18		20		23		26	
	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC
arboricola	-	2	-	-	-	2	-	-	-	1	-	-	-	-	-	-	-	8	-	-
baueri	1	2	1	-	-	4	-	-	-	1	-	2	-	-	-	-	2	1	-	-
biguttatus	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
crepuscularis	2	11	-	-	-	34	-	-	-	9	-	4	-	6	-	-	-	9	-	-
guttipennis	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-
haematopotus	1	-	-	-	-	-	-	-	2	-	-	1	-	1	-	-	5	-	-	-
obsoletus	-	3	-	-	-	3	-	-	-	2	1	2	2	3	-	-	8	-	-	-
snowi	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
stellifer	4	22	-	-	-	-	-	-	-	4	-	1	2	-	-	-	-	1	-	-
travisi	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
variipennis	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
venustus	5	4	-	-	-	1	-	-	-	-	-	1	-	1	-	-	1	1	-	-
	16	47	1	-	-	45	1	-	2	17	1	12	4	11	-	-	16	21	-	-

Totals: Woods 41

Poultry Coop 153

TABLE VII

Culicoides Trapped in Blacksburg Woods and Poultry Coop, September, 1960.

Culicoides Species	1		2		6		8		14		15		16		18	
	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC	W	PC
arboricola	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
baueri	-	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
crepuscularis	-	-	-	30	-	19	-	-	4	-	-	8	-	10	1	-
guttipennis	-	-	-	-	-	-	-	-	1	-	-	-	-	3	-	-
haematopotus	-	-	-	2	-	2	-	1	-	-	1	-	1	1	-	-
obsoletus	-	-	-	7	-	3	-	5	-	-	5	-	1	2	-	-
piliferus group	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
stellifer	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
variipennis	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
venustus	-	-	-	2	-	-	-	-	1	-	-	-	-	-	-	-
	-	1	-	43	-	24	-	7	6	-	6	9	3	16	1	-

Totals: Woods 16

Poultry Coop 100

TABLE VIII

Comparison of Results of Traps Located in Woods and in Poultry Houses.

Culicoides Species	Woods 1959	Woods 1960	Poultry Houses 1959	Poultry Houses 1960
arboricola	-	2	22	15
baueri	-	67	-	13
biguttatus	-	291	1	-
crepuscularis	-	10	1	200
chiopterus	-	3	-	-
debilipalpis	-	2	-	-
guttipennis	3	4	21	6
haematopotus	-	41	1	12
obsoletus	42	136	90	66
ousairani	-	1	1	3
piliferus group	1	21	2	-
snowi	-	2	-	-
spinosus	-	54	-	-
stellifer	-	180	2	52
travisi	-	321	2	-
varipennis	-	4	-	9
venustus	-	45	1	16
villosipennis	-	20	2	2
Totals	46	1204	146	394
	1250		540	

Effect of Weather: A thermo-humidigraph was operated more or less regularily near the battery-powered trap in the V.P.I. woods. However, the results obtained by comparing the total numbers of Culicoides taken by this trap to the temperature and relative humidity figures recorded by this instrument were too inconsistent to have any particular significance beyond the expected low catch on the coolest nights. Part of the difficulty was that on nights when the trap was powered by the same battery for two consecutive nights the battery ran down during the second night. This was not realized until more than half way through the season.

Larval Collections: The collection of larvae from a number of sites followed by the laboratory rearing of these specimens yielded the following results:

July 22, 1959, Tree hole - C. arboricola, 1 female; C. guttipennis, 15 males, 5 females; C. nanus, 8 males, 6 females.

July 31, 1959, Tree holes - C. arboricola, 3 males, 1 female; C. guttipennis, 43 males, 18 females; C. nanus, 1 female.

August 26, 1959, Tree hole - 8 C. guttipennis

April 1, 1960, Tree hole - 6 C. guttipennis

April 25, 1960, Tree hole - 58 C. guttipennis

April 28, 1960, Tree hole - 129 C. guttipennis

June 14, 1960, Tree hole - 6 C. guttipennis

June 14, 1960, Mud - 1 C. sp.

June 15, 1960, Muddy Water - 17 C. variipennis

June 15, 1960, Saline Water - 100's C. variipennis

June 28, 1960, Saline mud - 100's C. variipennis

July 6, 1960, Spring water - Negative

July 7, 1960, Barn runoff - Negative

July 15, 1960, Spring water - Negative

July 29, 1960, Tree hole - 1 C. guttipennis

August 5, 1960, Tree hole - 1 C. guttipennis

August 17, 1960, Site #1 - 44 C. variipennis

Site #2 - 4 C. variipennis

Site #3 - 349 C. variipennis

Site #4 - 177 C. variipennis

September 7, 1960, Saline water - 143 C. variipennis

August 8, 1960, Saline mud - 12,044+ C. variipennis

October 15, 1960, Saline mud - 418 C. variipennis

November 13, 1960, Site #1 - 11 C. variipennis

Site #2 - 0

Site #3 - 5 C. variipennis

Site #4 - 66 C. variipennis

As mentioned previously six crocks of mud collected at Saltville on September 8 were selected at random and carefully gone through in November to determine how many larvae were still in the crocks after the emergence of so many adults. The crocks contained 1,834, 537, 383, 40, 592, and 328 larvae in all instars. This is an average of 619 larvae per 500 cc. of mud remaining in the crocks after two months.

Rearing: As indicated above Culicoides were successfully reared from samples taken from a number of habitats. The results of our rearing program are summarized in Tables IX-XII and are presented in detail in the Appendix, Table B. In Table IX "Date" refers to the date the larvae were collected; "Number" means the number of larvae collected, if known; "Pupae" refers to the number of pupae removed from the pans to emergence cages; "Adults" refers to the number of adults which emerged; "Ave. Age" is the average ages of the adults when they died; "Oldest" means the age in days of the individual adult Culicoides that lived the longest time after it emerged; and "Last Adult" is the number of days after the larvae were collected that the last adult emerged.

In Tables X-XII "Days After Collection" is the number of days after the larvae were collected that the adults on this line emerged. "Number" refers to those adults whose ages were known exactly enough to compute the last two columns. "Ave. Age" and "Oldest" have the same meaning as above.

TABLE IX

Results of Pan Rearing 1959-1960.

Pan No.	Date	No.	Pupae	Adults	% Adults fr. Larv.	Ave. Age	Oldest	Last Adult
1	8/26/59	60	-	-	-	-	-	-
2	8/26/59	22	-	3	14	-	-	7
3	8/26/59	60	-	1	2	-	-	5
4	8/26/59	60	-	4	7	-	-	152
5	4/1/60	21	6	6	29	-	-	43
6	4/25/60	63	54	47	75	20.5	96	31
7	4/25/60	?	-	11	?	-	4	56
8	4/28/60	250	147	129	52	9	54	34
9	6/14/60	?	-	6	-	3.5	6	25
10	6/14/60	?	-	1	-	-	-	37
11	Same as above							
12	6/15/60	?	-	17	-	18.3	70	48
13	6/15/60	?	-	-	-	-	44	79
14-16	Same as above							
17	6/28/60	?	-	42	-	-	-	77
18	Same as above							
19	7/6/60	No Culicoides emerged						
20	7/7/60	No Culicoides emerged						
21	7/15/60	No Culicoides emerged						
22	7/29/60	1	1	1	100	3	3	3
23	8/5/60	?	-	1	-	-	-	39
24	9/7/60	?	-	143	-	-	-	38

TABLE X

Results of Crock Rearing of *Culicoides* Larvae

Collected June 15, 1960.

Days After Collection	Number	Ave. Age	Oldest
1	13	21.4	46
2	6	23.8	52
5	6	35.8	63
8	2	39.5	64
10	18	17.3	54
12	28	17.7	40
14	2	9.0	9
15	5	31.6	57
16	5	4.6	7
17	28	11.6	58
19	15	6.7	15
21	8	16.3	50
26	4	30.2	59
33	2	26.5	43
89	1	3.0	3

Results of Crock Rearing of *Culicoides* Larvae

Collected June 28, 1960.

Days After Collection	Number	Ave. Age	Oldest
3	8	5.9	-
8	8	17.0	58
11-20	No Data		
21	2	32.0	51
22	1	36.0	36
23	3	7.3	14
24	1	13.0	13
25	2	21.5	31
28	4	18.2	33
36	4	17.2	22
38	8	35.8	53
40	4	27.5	51
43	6	14.3	39
45	1	9.0	9
50	9	4.3	15
54	4	24.0	28
64	2	24.5	33
73	2	21.0	40
75	1	42.0	42
82	1	-	-

TABLE XII

Results of Crock Rearing of *Culicoides* Larvae

Collected August 17, 1960.

Days After Collection	Number	Ave. Age	Oldest
5	35	19.9	49
6	61	16.8	56
7	28	20.0	56
8	18	15.3	49
9	42	23.2	41
10	8	11.9	14
12	19	11.0	39
13	22	15.8	27
14	9	14.0	20
16	13	29.9	46
17	12	23.9	49
19	31	27.9	60
20	7	23.4	30
22	10	22.9	41
23	9	21.4	46
24	2	43.0	68
26	8	11.1	28
27	5	20.4	27

TABLE XII (Continued)

Results of Crock Rearing of *Culicoides* Larvae

Collected August 17, 1960.

Days After Collection	Number	Ave. Age	Oldest
28	4	36.2	47
29	1	2.0	2
33	1	22.0	22
34	5	19.2	55
36	5	21.8	43
41	2	13.5	24
44	1	31.0	31

Species in Each Habitat: As a result of the trapping and rearing program it has been possible to determine with a fair amount of assurance the habitat where the various species of Culicoides are to be found.

The trap located in the V. P. I. woods attracted all known Virginia species of Culicoides except borinqueni, footei, nanus, and paraensis. However, these have been taken in woods elsewhere in the state. Wirth (1951) and Wirth and Jones (1956) took footei in woods at Mt. Solon. The author took nanus from a tree hole in a woods and collected paraensis in a wooded area.

The larval collections revealed more specifically where certain species actually breed in nature. C. arboricola, C. guttipennis, and C. nanus were reared in tree holes. C. variipennis was found abundantly in all situations at the Saltville brackish water marsh. It was taken also from water which had accumulated in cattle hoof-prints in a pasture.

Some new locality records for Virginia Culicoides were established during this study. They are:

C. arboricola: Blacksburg, Elkton, Ferrum, New Castle (reared), Poplar Camp

C. baueri: Blacksburg

C. biguttatus: Blacksburg, Poplar Camp

C. chiopterus: Blacksburg

C. debilipalpis: Blacksburg, Ferrum

C. guttipennis: Blacksburg, Elkton, Ferrum, Poplar Camp

C. haematopotus: Blacksburg, Ferrum, Poplar Camp

C. nanus: Blacksburg, New Castle (all reared from tree holes)

C. obsoletus: Bent Mountain, Blacksburg, Elkton, Ferrum, Poplar Camp

C. ousairani: Blacksburg, Elkton, Ferrum, Poplar Camp

C. paraensis: Newport

C. piliferus group: Blacksburg, Poplar Camp

C. snowi: Blacksburg

C. spinosus: Blacksburg

C. stellifer: Blacksburg, Ferrum, Newport, Poplar Camp, Saltville

C. travisi: Blacksburg, Ferrum, Poplar Camp

C. variipennis: Blacksburg, Ferrum, Rural Retreat

C. venustus: Bent Mountain, Blacksburg, Elkton, Ferrum, Poplar Camp

C. villosipennis: Blacksburg, Elkton, Ferrum, Poplar Camp

Rate of Emergence: Accurate figures were not kept on the numbers of adults which emerged each day from the rearing vessels during the summer. The adults which emerged from the September, October, and November collections were counted, however. The graphs in Figures 1-3 give the results of these counts. Other rates of emergence are enumerated in Appendix, Table C.

The extensive trapping done at Blacksburg plus other collecting yielded information on the seasonal occurrence of certain species of

Culicoides as listed below:

C. Species	First Taken	Last Taken
arboricola	7/1/60	9/1/60
baueri	5/24/60	9/8/60
biguttatus	4/18/60	8/8/60
crepuscularis	5/24/60	9/18/60
chiopterus	6/22/60	6/24/60
debilipalpis	6/22/60	6/22/60
guttipennis	4/1/60	9/16/60
haematopotus	4/9/60	9/16/60
nanus	7/22/59	7/31/59
obsoletus	4/18/60	9/16/60
ousairani	6/20/60	8/8/60
paraensis	7/17/59	7/17/59
piliferus group	5/24/60	9/16/60
snowi	6/29/60	8/1/60
spinosus	5/24/60	7/1/60
stellifer	4/2/60	9/2/60
travisi	4/18/60	8/1/60
varipennis	4/18/60	11/13/60
venustus	5/24/60	9/14/60
villosipennis	6/22/60	8/8/60

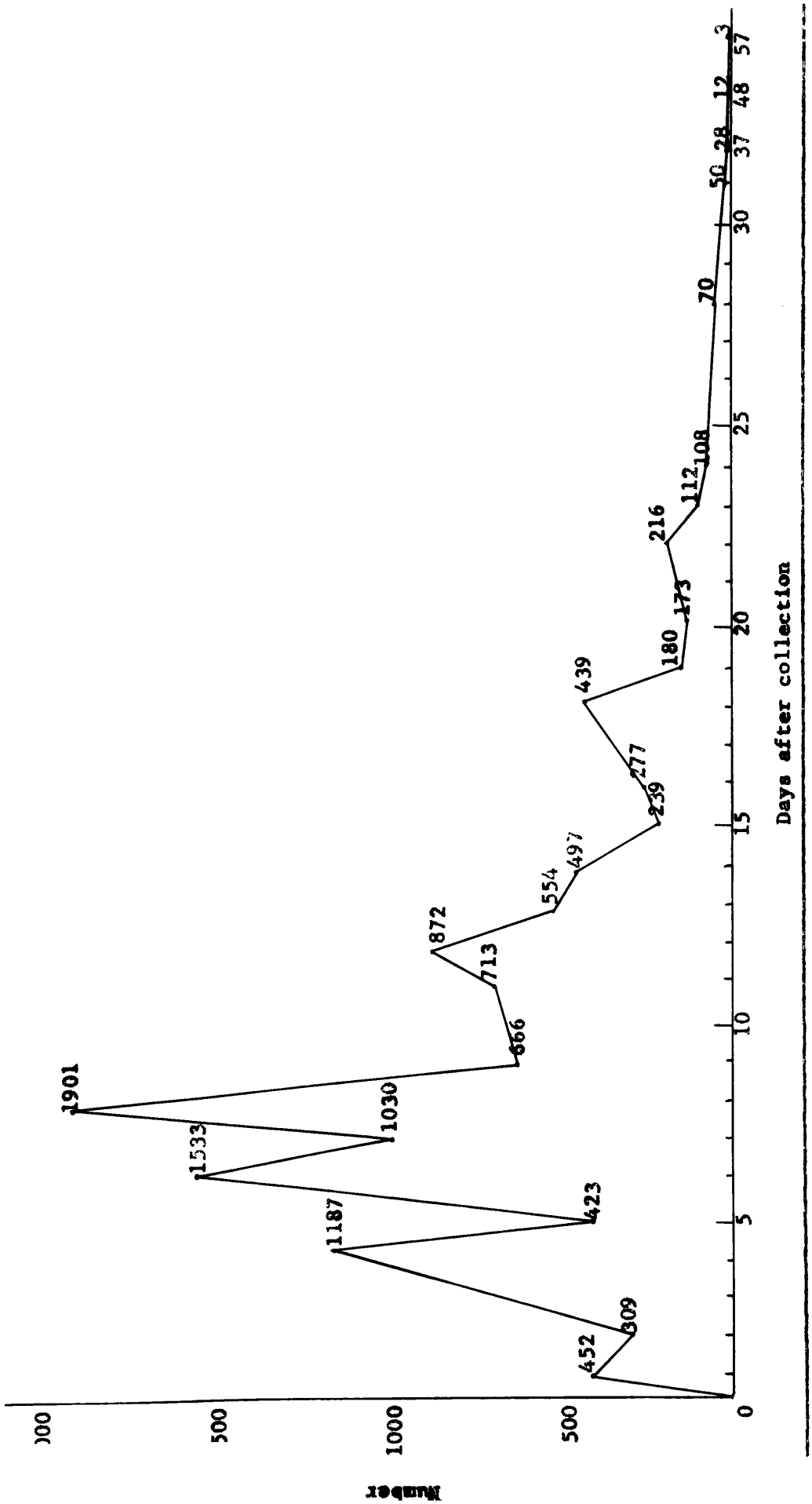

Figure 1. Emergence of Adults Collected as Larvae at Saltville, Virginia, September 8, 1960.

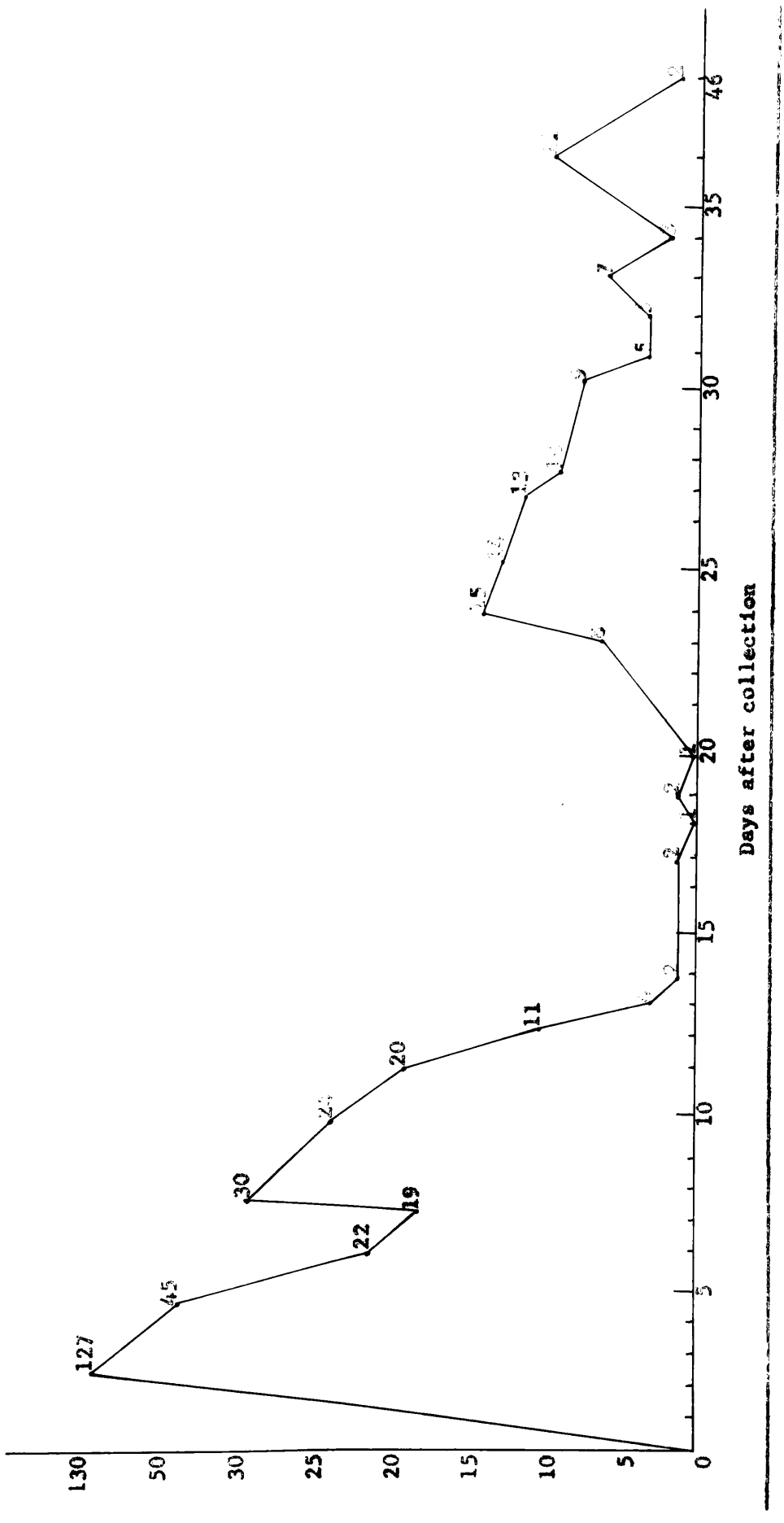

Figure 2. Emergence of Adults Collected as Larvae at Saltville, Virginia, October 15, 1960

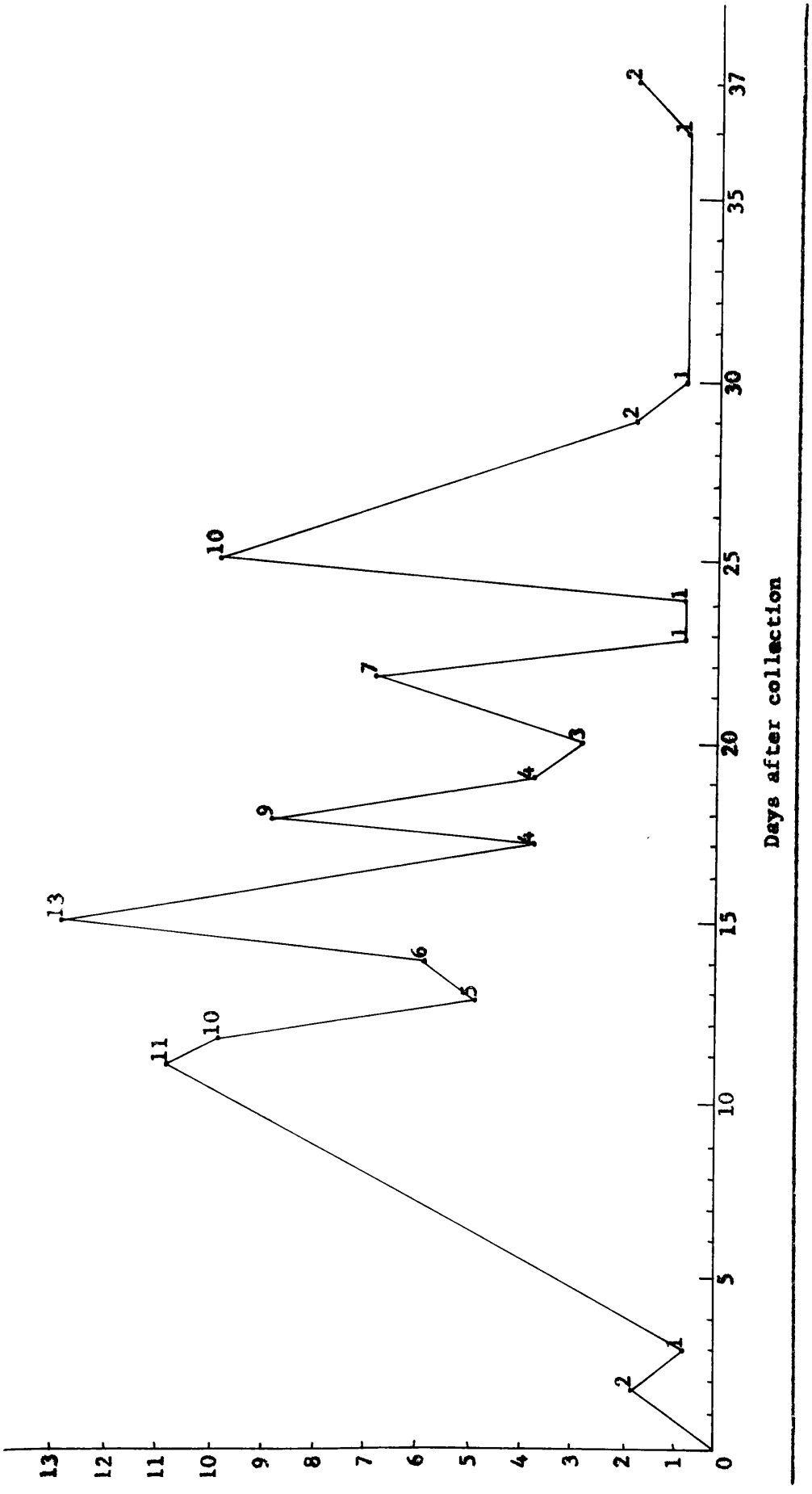

Figure 3. Emergence of adults collected as larvae at Saltville, Virginia, November 13, 1960.

Some indication of Culicoides abundance at Blacksburg may be obtained from Table XIII which summarizes the trapping there by months.

Effect of Temperature: As can be seen from the April 25 and 28, 1960 larval collections, air temperature does not influence larval abundance in tree holes, nor did it influence the larval collections at Saltville. Room temperature did not affect the emergence rates of adults which were reared in pans and crocks at V. P. I. Temperature seemingly had no apparent effect on trapping as discussed above. On the other hand, the constant temperature maintained by the incubator apparently contributed to the longevity of the Culicoides adults.

Longevity: The rearing technique gave the adults an anvironment conducive to longer life as can be seen in Tables IX-XII. Among those adults for which accurate records were kept, the longest any individual lived was 96 days. The average age for adults upon which records were kept was 27.7 days. It is felt the average would have been higher if so many insects had not died prematurely by adhering to wet surfaces in the cages. By actual count of the fate of adult variipennis collected as larvae it was found that 56 escaped, 332 were found dead under dry conditions, and 432 were found dead adhering to the water surface or wet paper toweling in their water pan.

TABLE XIII

Monthly Collection Totals of Culicoides Taken in Light Traps at Blacksburg, 1960.

Culicoides Species	April	May	June	July	Aug.	Sept.	Total
arboricola	-	-	-	3	13	1	17
baueri	-	2	59	3	14	2	80
biguttatus	1	111	163	15	1	-	291
chiopterus	-	-	3	-	-	-	3
crepuscularis	-	1	16	46	75	80	218
debilipalpis	-	-	2	-	-	-	2
guttipennis	-	-	-	3	3	4	10
haematopotus	10	2	19	4	10	8	53
obsoletus	6	3	89	57	25	23	203
ousairani	-	-	1	3	-	-	4
piliferus group	-	6	14	-	-	1	21
snowi	-	-	1	-	1	-	2
spinosus	-	14	38	2	-	-	54
stellifer	2	-	187	8	34	1	232
travisi	3	123	177	14	2	-	319
varipennis	2	1	1	5	3	1	13
venustus	-	26	15	3	14	3	61
villosipennis	-	-	18	4	-	-	22
Totals	24	289	803	170	195	124	1605

TABLE XIV

Feeding of *Culicoides* on Chickens.

Date	Part of Chicken	Light	*Culicoides* Species	Age (Days)	Number Engorged
5/9/60	Head	Dim	*guttipennis*	6	None
5/10/60	Head	Dim	*guttipennis*	7	None
6/21/60	Hock	Dim	*variipennis*	4	"Some"
6/24/60	Hock	Bright	*variipennis*	2-8	"Many"
6/29/60	Hock	Medium	*variipennis*	4	?
6/30/60	Thigh	Bright	*variipennis*	3	"Almost All"
7/4/60	Thigh	Bright	*variipennis*	4,6	"Some"
7/8/60	Thigh	Bright	*variipennis*	4,7,10	None
7/18/60	No Data		*variipennis*	13,14,17	None
7/19/60	No Data		*variipennis*	5,6,8	"Some"
7/28/60	Hock	Bright	*variipennis*	15,19	11 "Most
	Hock	Bright	*variipennis*	13,14	3 would
	Hock	Bright	*variipennis*	6	2 not
	Thigh	Bright	*variipennis*	10	3 feed"
7/30/60	Head	Bright	*variipennis*	10,11,12	31
	Foot	Bright	*variipennis*	11,16,18	2
	Head	Bright	*variipennis*	4,7,8,9	20 of 88
8/1/60	Head	Bright	*variipennis*	1-12	12
9/15/60	Head	Bright	*variipennis*	No Data	92
9/17/60	Head	Bright	*variipennis*	No Data	410
9/19/60	Head	Bright	*variipennis*	No Data	181

Infectious Synovitis Study

Feeding: The technique which was employed most successfully utilized a pint sized ice cream carton and a hardware cloth cone slipped over the chicken's head, as discussed earlier. A summary of all feeding attempts is presented in Table XIV.

Transmission Experiments, 1959: The use of live-trapped, engorged female Culicoides of various species for injection into healthy chickens to demonstrate the presence of the infectious synovitis agent gave negative results. However, it must be kept in mind that the chickens upon which these Culicoides had fed had been receiving aureomycin in their feed.

Transmission Experiments, 1960: All of this year's experiments attempted to demonstrate the viability of the synovitis agent in Culicoides. As noted above, C. guttipennis females could not be induced to feed upon a chicken. Therefore, a promising species was used (C. variipennis), which had already been incriminated as a vector of a virus disease in Texas (Price and Hardy, 1954).

A sufficient quantity (767) of engorged females were obtained to test them as potential vectors. The results in all cases were negative.

Other Observations

C. variipennis apparently does not regularly feed on man in nature. The author was bitten less than half a dozen times during the course of his work in the field and in the laboratory. Inquiry

at Saltville indicated mosquitoes (mostly Aedes sollicitans), not punkies, were the principal pests.

On January 29, 1960, a Culicoides larva which had been collected on August 26, 1959, from a tree hole was seen in a piece of rotting wood.

C. guttipennis apparently overwinters as a larva as indicated by observations on pan #4. The larvae were collected on August 26, 1959. On January 29, 1960, four dead guttipennis adults were removed. On April 4, 1960, a living guttipennis female was removed.

The larval food is not well known, but on May 3, 1960, the following was noted in pan #8. "Observed two or three larvae in action apparently eating exuvium from one of adults that had emerged".

One of the hazards all Culicoides which were reared in the laboratory had to overcome was escape from being trapped in the surface tension of the water from which they emerged. For instance, 12,044+ adults were reared and safely removed from the crocks of the September 8, 1960 collection, but at the time of discard 1551 dead and decaying adults were on the water - trapped by the surface tension. How this occurs may be seen from some observations taken on an adult which emerged in pan #8 on May 6, 1960. Notes taken at the time follow:

2:00: Emerged from exuvium - white, eyes dark; immediately walked on water.

2:02: To edge (of pan). Right wing on top left wing; right front foot stuck to something.

2:05: Struggling to free foot; left foot like lever; free; did not rest; cleaned rear feet.

2:06: Some darkening of abdomen and dorsal thorax; also joints.

2:08: Thoracic spines dark; pattern indistinct.

2:10: Torus dark; not moving (standing on water surface). Entire rear tarsus parallel to water surface and on it.

2:17: Moved; saw halteres jerk anterior-posterior.

2:20: Walking jerkily on water.

2:21: Slight movement of antennae and head.

2:23: When he struggles to walk halteres move posteriorly; tip of abdomen touches water; head erect.

2:35: Left wing on top of right wing; seems to be having difficulty lifting rear tarsi from water surface.

2:37: Lowered head briefly.

3:17: Seems to be trapped on surface as were its siblings. Right wing tip and right legs along surface and adhering to it. Put in (cage) #14.

3:35: Dark now, but hasn't flown yet.

It was also observed that engorged females lived no longer in the holding cages than unfed females who had to subsist only on raisins and water.

VI. CONCLUSIONS

A number of species of Culicoides are found rather commonly in Virginia as indicated by the trapping and rearing studies reported herein. These may be listed in order of decreasing abundance based upon trapping results. The figures indicate the total numbers trapped at all localities during both 1959 and 1960. C. obsoletus (2078), C. crepuscularis (351), C. arboricola (336), C. travisi (326), C. biguttatus (292), C. stellifer (282), C. guttipennis (254), C. villosipennis (128), C. venustus (94), C. baueri (81), C. haematopotus (62), C. spinosus (54), C. piliferus group (24), C. ousairani (22), C. variipennis (16), C. debilipalpis (5), C. chiopterus (3), C. snowi (2), and C. paraensis (1). In addition, 15 C. nanus were reared. The only species known to occur in Virginia which were not taken in this study were C. borinqueni and C. footei.

All of the species taken during this study except C. paraensis have been found at Blacksburg, hence it is believed that if as concentrated a trapping and rearing program were to be pursued at other suitable localities throughout the state additional locality records for every species would be obtained.

The trapping program revealed that the following species of Culicoides can be found associated with poultry and probably feed upon them in Virginia: arboricola, baueri, biguttatus, crepuscularis, debilipalpis, guttipennis, haematopotus, obsoletus, ousairani, piliferus group, stellifer, travisi, variipennis, venustus, and villosipennis. The relatively small numbers of baueri, biguttatus,

debilipalpis, guttipennis, haematopotus, ousairani, piliferus group, travisi, variipennis, venustus, and villosipennis taken in poultry houses would tend to eliminate them as potential vectors of infectious synovitis. This leaves just arboricola, crepuscularis, obsoletus, and stellifer. C. obsoletus was found to be almost as abundant (2078:2333) as all other species trapped, so it appears to be the most likely culicoid vector of infectious synovitis yet to be investigated.

Trapping at Elkton tended to support the idea that more Culicoides would be found in poultry houses nearer to a woods than those farther from a woods. Each room in the house nearer the woods yielded almost twice as many (234 and 285:142) Culicoides as the entire house away from the woods. This was generally true at Ferrum and Poplar Camp, although exact figures were not kept.

Temperature and relative humidity had little appreciable effect on the results of our trapping program. The same may be said for the larval collecting and rearing undertakings.

These studies further verified certain known facts about the habitats of certain species of Culicoides. C. arboricola, guttipennis, and nanus were found to be tree hole breeders. C. variipennis was taken from contaminated water and saline mud and water. The larvae of C. variipennis were found to be most abundant in the top 1/2 to one inch of saline mud provided it was covered by about 1/4 inch of water. Under these conditions as many as 2000 larvae per 500 cc. of mud can be collected.

It was found that large numbers of Culicoides may be reared simply by placing the substrate in earthenware crocks with an emergence cage on top. If the substrate is kept wet, adults will continue to emerge for two or more months. However, they will emerge in largest numbers four to eight days after collection.

The technique of keeping the adults in holding cages fashioned from ice cream cartons in a darkened incubator at 75° F. proved to be very successful. An improved method of providing a constant supply of water would further decrease the mortality.

By running the traps well into late summer the known period of activity of several species was extended. Some were taken on the last day the traps were in operation which indicates they would be active still longer. Additional trapping should corroborate this assumption.

The results indicate when certain species reach their peak of abundance at Blacksburg. For instance: May - venustus; June - baueri, biguttatus, haematopotus, obsoletus, spinosus, stellifer, travisi, and villosipennis; August - arboricola; September - crepuscularis. A few species were found every month: haematopotus, obsoletus, stellifer, and variipennis. C. guttipennis and venustus probably breed all summer. These six species undoubtedly have two or three generations a year in our area. Possibly some of the other species do also.

The head feeding technique employed was effective, but should be improved upon. Insects two to four days old feed most readily. C. variipennis has now been shown to feed readily on chickens in bright light, but we must conclude that this species is not a vector of infectious synovitis, nor is C. guttipennis. C. obsoletus should be tested to determine if it is a vector of infectious synovitis in poultry in Virginia.

VII. SUMMARY

Culicoides are known vectors of viral and filarial diseases in birds and mammals, including man. Therefore, a survey of these insects in Virginia was undertaken in an attempt to determine their distribution in the state and their relationship to poultry.

Trapping and rearing studies added four species to the published Culicoides fauna of Virginia. These are C. chiopterus, a European species; C. debilipalpis and C. paraensis, both neotropical species; and C. ousairani, a mid-western species. Twenty-two species of this genus are now known to be in Virginia. Twenty of these were taken in the course of this study. Nine Virginia localities yielded various species of Culicoides with 19 being found at Blacksburg.

All specimens were preserved in 70 per cent alcohol and later mounted on microscope slides.

Weather conditions and temperature had no appreciable effect on trapping and rearing results.

All species of Virginia Culicoides have been taken in wooded habitats where they apparently breed. Fifteen species are found in poultry houses, but only four of these were abundant enough to pose a threat. C. obsoletus is the most abundant Virginia species and hence it is suggested that tests be conducted to determine if this species is a vector of infectious synovitis.

Some species were reared in the laboratory with the most successful technique being one which utilized an earthenware crock to hold the larval substrate and an ice cream carton emergence cage to capture the adults as they emerged. The adults were kept in

an incubator at 75° F. They were given only raisins and water, yet lived as long as 96 days. The average age at death was about 28 days.

It was found that the greatest number of adults emerged four to eight days after the larvae were collected. They fed most readily on the heads of chickens when they were two to four days of age. C. variipennis was found to be extremely abundant near the surface of wet, saline mud at Saltville, Virginia. It was used in transmission studies.

Live-trapped Culicoides did not transmit infectious synovitis to healthy chickens. Neither did laboratory-reared punkies (which had fed upon diseased chickens) when held at varying intervals, macerated, and injected into healthy chickens.

VIII. VITA

Donald Howard Messersmith

The author of this paper was born in Toledo, Ohio, on the seventeenth of December, 1928, the son of Howard Goodwin and Elizabeth Helen Messersmith. He attended the public schools of that city, graduating from DeVilbiss High School in June, 1947. He matriculated at the University of Toledo in the fall of 1947. In June, 1951, he was awarded the B. Ed. degree with majors in Education and Biology. He entered the University of Michigan in the fall of 1951 and received the M.S. degree in Biology in June, 1953. He was conscripted into the United States Army in August, 1953, and served at the Army Medical Center in Washington, D. C. until June, 1955. At various times he has travelled throughout North America including Alaska. He has led tours in Europe and travelled independently including a trip in 1955 to parts of the Middle East. From September, 1956, until June, 1957, he taught science in the Washington public schools. He was married June 15, 1957, to Margarita Park Sherertz; they have one child, Mary Helen, born November 23, 1958. During the academic year 1957-58, he was a research assistant at Virginia Polytechnic Institute. Since September, 1958, he has been a member of the faculty of Radford College in the Biology Department. He is a member of the Entomological Society of America, Phi Sigma, Beta Beta Beta, A.I.B.S. and a number of other scientific and professional organizations.

Donald H. Messersmith

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X. APPENDIX

TABLE A

Results of Larval Collections and Rearing from Treeholes, 1959

Location	Date	Species	Males	Females
Newport	7/22	arboricola	-	1
		guttipennis	15	5
		nanus	8	6
Blacksburg	7/31	arboricola	3	1
		guttipennis	43	18
		nanus	-	1
Blacksburg	8/26	C. spp.	from 202 larvae, 8 adults emerged prior to 1/29/60.	

TABLE B

Results of Larval Collections and Rearing, 1960

Locality & Date	Pan No.																			Totals
Blacksburg	5	April	30	May	3	4	6	9	10	12	14	16								
Tree Hole	(21 larvae)	Pupae	-		1	3	-	1	1	-	-	-								6
(April 1)		Adult	-		-	1	1	2	-	1	1	-								6
		Dead	1̲*		-	-	-	1	-	1	4	1								8
Blacksburg	6	April	26	28	29	30	May	2	3	4	5	6	7	9	10	11	12	16	18	
Tree Hole	(63 larvae)	Pupa	1	2	2	4		3	5	1	1	1	1	2	-	-	9	-	-	32
(April 25)		Adult	-	-	-	-		1	4	5	1	1	6	2	1	3	4	-	1	29
Cage #14		Dead	-	-	-	-		-	1̲	2	-	-	2	1	-	3	-	8	2̲	19
		June	3	13	20	July	9													
		Dead	1̲	6̲	2		1													10
		May	10	11	12	14	16	17	18	20	June	6	17	22	27	Aug.	12	22		
Cage #7	6	Pupa	8	3	-	-	-	-	-	-		-	-	-	-		-	-		11
		Adult	-	-	1	1	8	-	1	-		-	-	-	-		-	-		11
		Dead	-	-	-	-	2	1	1	1̲		1	1̲	1	1		1	1		11
		May	13	25																
Cage #5	6	Pupa	4	4 died																4
		May	14	18	20	24	28													
Cage #1	6	Pupa	1	-	1	-	-													2
		Adult	-	1	-	1	-													2
		Died	-	-	1	-	1													2

* Underlined indicates the insect escaped.

TABLE B

Results of Larval Collections and Rearing, 1960 (Continued)

Locality & Date	Pan No.										Totals
		May	16	17	20	25					
Cage #2	6	Pupa	2	-	-	-					2
		Adult	-	2	-	-					2
		Died	-	-	1	1					2
		May	19	24	June	6	25				
Cage #104	6	Pupa	2	-		-	-				2
		Adult	-	2		-	-				2
		Died	-	-		1	1				2
		May	19	24	June	6	25				
Blacksburg	6	Pupa	2	-		-	-				2
Tree Hole		Adult	-	2		-	-				2
(May 25)		Dead	-	-		1	1				2
		May	21	26	June	4					
Cage #13	6	Pupa	1	-		-					1
		Adult	-	1		-					1
		Dead	-	-		1					1
		May	13	16	17	18	June	1	13	20	
Cage #102	7	Pupa	-	-	-	-		1	-	-	1
		Adult	1	5	1	1		-	1	1	10
		Dead	1	5	1	1		-	1	1	10
		May	18								
Cage #3	7	Adult	1								1

TABLE B

Results of Larval Collections and Rearing, 1960 (Continued)

Locality & Date	Pan No.													Totals
		May	2	3	5	6	7	9	June	30				
Newport	8	Pupa	15	-	-	-	-	-		-				15
Tree Holes	(250 larvae)	Adult	-	1	1	8	7	-		-				17
(May 28)		Dead	-	-	-	6	9	1		1				17
Cage #6														
		May	3	4	5	6	7	9	10	12	13	14	28	June 13
Cage #100	8	Pupa	29	21	-	-	-	5	died	-	-	-	-	- 50
		Adult	3	3	1	12	10	11	-	-	-	-	-	- 40
		Dead	-	-	-	1	-	24	8	-	2	4	1	1 43
		May	5	6	7	9	10	11	12	13	14	16	17	19
Cage #101	8	Pupa	9	13	17	-	-	-	-	-	-	-	-	- 39
		Adult	-	-	-	4	10	19	1	1	-	-	-	1 36
		Dead	-	-	-	4	3	5	8	4	2	6	2	- 34
		June	14	29	July	5								
		Dead	1	2		1								4
		May	9	10	11	12	13	14	16	17	18	19	20	27
Cage #105	8	Pupa	15	-	-	-	-	-	-	-	-	-	-	- 15
		Adult	-	2	5	3	3	-	-	-	-	-	-	- 13
		Dead	-	-	-	-	3	1	2	1	2	1	1	1 12
		May	10	14	16									
Newport	8	Pupa	9	-	-									9
(April 28)		Adult	-	5	4									9
Cage #106		Dead	-	4	5									9

TABLE B

Results of Larval Collections and Rearing, 1960 (Continued)

Locality & Date	Pan No.									Totals
		May	11	14	16	17	18	19	July 4	
Cage #3	8	Pupa	2	-	1	1	-	-	-	4
		Adult	-	2	-	1	1	-	-	4
		Dead	-	1	1	-	-	1	1	4
		May	12	14	25					
Cage #103	8	Pupa	5	-	4	dead				5
		Adult	-	1	-					1
		Dead	-	1	-					1
		May	16	21	26					
Cage #11	8	Pupa	2	-	1	dead				2
		Adult	-	1	-					1
		Dead	-	1	-					1
		May	19	24	26		26	30		
Cage #12	8	Pupa	2	-	1	dead	1	-		3
		Adult	-	1	-		-	1		2
		Dead	-	1	-		-	1		2
		May	23	24	25	26	27			
Cage #102	8	Pupa	4	-	-	-	-			4
		Adult	-	1	2	1	-			4
		Dead	-	1	-	2	1			4
		May	25	27		June	1			
Cage #5	8	Pupa	2	-			-			2
		Adult	-	1			1			2
		Dead	-	-			2			2

TABLE B

Results of Larval Collections and Rearing, 1960 (Continued)

Locality & Date	Pan No.												Totals
Newcastle		June	23	24	30	July	7	9	11	August	2		
Tree hole	9	Pupa	-	-	-		-	-	-		1		1
Litter		Adult	1	2	-		1	1	-		-		5
(June 14)		Dead	-	1	1		-	-	1		-		3
Cage #1													
		June	29	July	4								
Newcastle	9	Adult	1		-								1
(June 14)		Dead	-		1								1
Cage #8													
		July	21										
Newcastle Mud	10, 11	Adult	1										1
(June 14)													
		June	20	22	23	24	25	July	1	7	20		
Rural Retreat	12	Pupa	2	-	4	-	-		-	-	-		6
Muddy Water		Adult	-	2	2	2	2		4	1	1		14
(June 15)		Dead	-	-	-	-	1		-	-	1		2
Cage #25													
		July	26	August	2	September	8	12					
Cage #34	12	Adult	2		1		-	-					3
		Dead	-		-		2	1					3

TABLE C

Emergence and Longevity of Culicoides Collected at Saltville,

June 15, 1960

Cage Number	Date Emerged	Total Died				
		June	July	August	Sept.	Oct.
202	6/16/60	-	9	1	-	-
22	6/17/60	-	-	2	-	-
21	6/22/60	-	4	2	-	-
24	6/23/60	-	1	1	-	-
25	6/25/60	-	14	4	-	-
200	6/27/60	1	24	3	-	-
29	6/29/60	-	2	-	-	-
26	6/30/60	-	2	3	-	-
30	7/1/60	-	5	-	-	-
10	7/2/60	-	17	2	-	-
23	7/4/60	-	15	-	-	-
31	7/6/60	-	6	2	-	-
210	7/7/60	-	10	2	-	-
32	7/11/60	-	4	1	1	-
23A	7/13/60	-	3	1	-	-
14	8/31/60	-	-	-	1	-
14	9/3/60	-	-	-	1	-
38	9/12/60	-	-	-	-	1
Pans 13, 14, 15, 16		-	3	-	8	7

TABLE D

Emergence and Longevity of Culicoides Collected at Saltville, June 28, 1960

Cage Number	Date Emerged	No.	Total Died			
			July	August	Sept.	Oct.
30	7/1/60	-	8	-	-	-
31	7/6/60	-	5	2	-	-
303	7/9/60	-	Used for feeding experiments			
304	7/11/60	-	15	Used for feeding		
305	7/12/60	-	10	-	-	-
306	7/13/60	-	7	Used for feeding		
307	7/14/60	20	Used for feeding experiments			
308	7/15/60	-	3	Used for feeding		
309	7/18/60	-	Used for feeding experiments			
310	7/19/60	-	Used for feeding experiments			
311	7/19/60	30	5	Used for feeding		
312	7/20/60	-	Used for feeding experiments			
313	7/21/60	80	2	Used for feeding		
314	7/22/60	85	Used for feeding experiments			
315	7/23/60	25	Used for feeding experiments			
316	7/26/60	61	Used for feeding experiments			
317	8/1/60	-	Used for feeding experiments			
320	8/4/60	-	-	5	-	-
321	8/5/60	11	-	2	6	-
322	8/8/60	5	-	3	1	-

TABLE D

Emergence and Longevity of Culicoides Collected at Saltville,

June 28, 1960 (Continued)

Cage Number	Date Emerged	No.	Total Died			
			July	August	Sept.	Oct.
324	8/13/60	15	-	4	?	-
325	8/13/60	9	-	1	-	-
326	8/18/60	9	-	9	-	-
37	8/22/60	3	-	1	2	-
327	9/3/60	2	-	-	1	1
328	9/10/60	2	-	-	1	1
329	9/12/60	1	-	-	-	1

TABLE E

Emergence and Longevity of *Culicoides* Collected at Saltville, August 17, 1960

Cage Number	Date Emerged	Number	Total Died		
			September	October	November
400	8/18-9/2	51	33	2	-
401	8/18-9/2	73	56	5	-
402	8/18-9/2	51	24	4	-
403	8/18-9/2	49	17	1	-
404	8/18-9/2	53	31	11	-
405	8/18-9/2	13	8	-	-
406	8/18-9/2	34	17	2	-
407	8/18-9/2	43	22	-	-
408	8/18-9/2	26	9	-	-
409	8/18-9/2	45	6	7	-
410	9/3/60	30	8	4	-
411	9/5/60	43	12	18	1
412	9/6/60	12	2	5	-
413	9/8/60	22	5	5	-
414	9/9/60	10	6	2	-
415	9/10/60	2	1	-	1
416	9/12/60	9	7	1	-
417	9/13/60	4	1	3	-
418	9/14/60	4	-	4	-
419	9/15/60	1	1	-	-

TABLE E

Emergence and Longevity of Culicoides Collected at Saltville,

August 17, 1960 (Continued)

Cage Number	Date Emerged	Number	Total Died		
			September	October	November
420	9/19/60	1	-	1	-
421	9/20/60	5	3	1	1
422	9/23/60	5	2	2	1
423	9/27/60	2	1	1	-
424	9/30/60	1	-	1	-

TABLE F

Miscellaneous Collections, 1960

Pan No.	Locality & Date	
19	Blacksburg Pasture Spring (July 6)	July 21 2 Chironomidae emerged
20	Blacksburg Barn Runoff (July 7)	July 13-27 Psychodidae emerged
21	Vesuvius Spring (July 15)	July 22 to August 13 4 Chironomidae emerged
22	Blacksburg Stump (July 29)	August 1 1 Culicoides emerged, died Aug. 4
23	Newport Tree Hole (August 5)	September 13 1 Culicoides emerged

TABLE G

Miscellaneous Larval Collections, 1960

Locality & Date	Emergence Dates												
	Sept.	12	13	14	17	20	21	22	23	24	26	27	28	30
Saltville Algal Pond No. Adults (Sept. 7)		12	13	26	43	17	4	9	3	1	7	2	1	1
	October	1	2	9	15									
		1	1	1	1									
	Sept.	9		10		12		13		14		15		16
Saltville Mud Flat No. Adults		452		309		1187		423		1533		1031		1901
To Cage # (Sept. 8)		500		501		502		503		504		505		506
	Sept.	17		19		20		21		22		23		24
No. Adults		666		713		872		554		497		239		277
To Cage #		507		508		509		510		511		512		513
	Sept.	26		27		28		30						
No. Adults		439		180		173		216						
To Cage #		514		515		516		517						
	Oct.	1		2		4		9		15		18	Nov.	3
No. Adults		112		108		70		50		28		12		3
To Cage #		518		519		520		521		522		523		-

TABLE H

Emergence of Adult Culicoides Collected at Saltville,

October 15, 1960

	Oct. 16-19	20	21	22	24	25	26	27	28	29
Adults	127	45	22	19	30	24	20	11	4	2

	Nov. 1	2	3	4	5	7	8	9	11	12	14	15	16	17
Adults	2	1	2	1	*	8	15	14	13	10	9	5	5	7

	Nov. 18	20	21	25	27	28	30	Dec. 1	2	3	7
Adults	3	11	2	2	1	3	1	4	3	1	1

* Added Water

TABLE I

Emergence of Adult Culicoides Collected at Saltville

November 13, 1960

Site No.	November							December										Totals
	15	16	25	26	27	28	30	1	2	3	5	6	7	8	12	13	19	20	
1	2	1	2	-	1	2	1	1	1	-	-	-	-	-	-	-	-	-	11
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
4	-	-	3	5	5	11	3	8	3	3	7	1	1	10	2	1	1	2	66

TABLE J

Other Insects Captured During the *Culicoides* Trapping Program, 1959, 1960

Orthoptera	7	Diptera	
Plecoptera	45	**Tipulidae**	3340
Dermaptera	12	**Anisopodidae**	290
Psocoptera	155	**Psychodidae**	28090
Hemiptera	405	**Chironomidae**	20957
Homoptera	8995	**Ceratopogonidae (Exclusive of *Culicoides*)**	28104
Neuroptera	73	**Culicidae**	475
Coleoptera	2107	**Dixidae**	257
Mecoptera	2	**Scatopsidae**	49
Trichoptera	1042	**Mycetophilidae**	5929
Lepidoptera	20920	**Sciaridae**	4354
Hymenoptera	10154	**Cecidomyidae**	112,332
		Brachycera	8059
	Total	256,153	

ABSTRACT

of

STUDIES ON CULICOIDES (DIPTERA: CERATOPOGONIDAE)

AND THEIR RELATIONSHIP TO INFECTIOUS SYNOVITIS

IN POULTRY IN VIRGINIA

Donald H. Messersmith

A Dissertation submitted to the Graduate Committee

of Virginia Polytechnic Institute in Partial Fulfillment

of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

ENTOMOLOGY

This investigation was concerned with determining the relationships of Culicoides to poultry in Virginia while studying their role as potential vectors of infectious synovitis. In addition, a survey of Culicoides in Virginia was made to study their distribution, bionomics and life histories. The insects were trapped in the field, collected as larvae, and reared in the laboratory.

Using light traps in chicken coops at Ferrum, Virginia in 1959, it was found that the following species of Culicoides were present: arboricola, crepuscularis, debilipalpis, guttipennis, haematopotus, obsoletus (most abundant), ousairani, stellifer, travisi, variipennis, venustus, and villosipennis. During the same summer, traps at Poplar Camp took many of the above species plus biguttatus and the piliferus group. That same year a single specimen of C. paraensis was taken while biting the author.

Light trapping in 1960 was done at Blacksburg about three nights a week for six months. Other trapping was done at Elkton, Newport and Saltville. At Blacksburg and Elkton traps were operated both in the woods and in poultry houses. The 1960 trapping activities revealed the following species were present: arboricola, baueri, biguttatus, crepuscularis, chiopterus, debilipalpis, guttipennis, haematopotus, obsoletus, ousairani, piliferus group, snowi, spinosus, stellifer, travisi, variipennis, venustus, and villosipennis. All but chiopterus, debilipalpis, snowi, and spinosus have been taken in poultry houses. In 1960 crepuscularis was the most abundant species taken in poultry houses and travisi was the most numerous species in the woods.

It was found that more Culicoides were taken in poultry houses located near a woods than in ones farther from a woods.

Trapping revealed that Culicoides were most abundant at Blacksburg in June, with May being the next most favorable month. The following species were found every month: haematopotus, obsoletus, stellifer, and variipennis.

All specimens were preserved in 70 per cent ethyl alcohol and later mounted in phenol-balsam on microscope slides.

Apparently the weather had little influence on the trapping results.

During 1959 and 1960 larvae were collected in a number of situations, but mostly from rot holes in trees and soft mud. Tree-hole collections produced specimens of C. arboricola, guttipennis, and nanus. Mud and muddy water produced C. variipennis. The latter were extremely abundant in saline mud at Saltville, there being found as many as 2000 larvae per 500 cc. of mud. These were used in the infectious synovitis transmission experiments.

The larvae were reared in pans and crocks containing their original substrate. After emergence they were held in cages fashioned from round ice cream cartons, and kept in an incubator at 75° F. and approximately 40 per cent relative humidity. Under these conditions the adults lived an average of 27.7 days after emergence with the longest time being 96 days. The adults emerged in largest numbers four to eight days after collection.

In 1959 attempts were made to transmit infectious synovitis to healthy chickens by injecting them with macerated, engorged Culicoides which had been live-trapped in poultry houses containing chickens infected with the disease. These attempts were unsuccessful.

In 1960 specimens of C. varilipennis were induced to feed upon chickens injected with the agent of infectious synovitis. These were later macerated at varying intervals and injected into healthy chickens. No transmission was demonstrated. It was found that the insects fed most readily on the heads of chickens when the insects were two to four days old.