through the thorax of virgin females it provides a marked oviposition stimulus.

Action of the accessory gland in other Diptera is under current investigation. A chemical analysis of the glandular substance is also proposed.

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# TECHNIQUES FOR OBTAINING VIABLE EGGS OF *LEPTOCONOPS BEQUAERTI* KIEFFER, *CULICOIDES FURENS* POEY, AND *CULICOIDES BARBOSAI* WIRTH AND BLANTON, (DIPTERA: CERATOPOGONIDAE)

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Jamaica is an extremely beautiful island with a thriving tourist industry. However, the welfare of tourism is constantly threatened by the activities of various biting insects, notably certain mosquitos and some species of biting Ceratopogonidae. The latter play such an important part in these considerations that a research unit was established in 1959 with the specific task of investigating the biology of these insects.

It soon became apparent that the problems were being caused almost entirely by the three species named in the title of this paper. *Leptoconops bequaerti* Kieffer bites throughout the day in strong sunlight and quite high winds (Kettle and Linley, 1960a), and is in fact a particular nuisance as it breeds in white sand of the type found on the most attractive beaches. In fact, one well respected hotel in the Montego Bay area was breeding its own problem beneath the beach shelters provided for visitors.

*Culicoides furens* Poey and *C. barbosai* Wirth and Blanton are active mainly at dawn and dusk, and are associated with the coastal mangrove swamps. There are large areas of such swamp in Jamaica, often adjacent to development projects already completed or to localities with po-

tential for development. Many small fishing villages are to be found close to the swamps and a good deal of suffering must result from the activities of *Culicoides*.

The techniques to be described here were developed in conjunction with more complete studies on the ovarian cycle in the three species. It is hoped that they make some contribution towards the establishment of self-maintaining laboratory colonies, whilst in addition it is always useful to have viable eggs available for other experiments. The techniques are successful only because females of all three species are mated when they alight to feed in the field. The exact proportion of mated individuals in each case has not been determined, but in *L. bequaerti* it constitutes a very high fraction of the population. This is also true of *C. barbosai,* which reverses an earlier opinion cited by Kettle (1962). *C. furens* appears to be the most disappointing in this respect, but it is nevertheless possible to obtain quite good numbers of eggs from wild-caught females. The methods appropriate to the three species are considered in turn below.

*Leptoconops bequaerti.* This is an easy insect to collect as it bites throughout the daylight hours. It prefers to feed on the leg (Kettle and Linley, 1960a), and a convenient catching position is to sit on a low box or stone so that the legs can easily be seen. A small aspirator should be used to capture the females, constructed in such a way that the receptacle can be quickly changed when about 60 individuals have been taken. Small specimen tubes (3 x 1 inch) are very suitable as containers and are conveniently small for handling later on. It is not advisable to collect more than 60 females per tube for reasons that will become apparent later, and care should be taken to suck the insects into the tube gently in order to minimize mechanical damage. Changing the aspirator tube provides no problem as *L. bequaerti* females are strongly attracted to light and will fly to the sunlit end of the tube during the changeover. In a good locality it is possible to collect over 500 females in 15 minutes in this way.

As soon as each tube has been stocked with flies it should be sealed, preferably with a rubber bung, and placed in a large thermos flask containing a small quantity of ice well insulated from the tubes by an ample thickness of cotton wool. On return to the laboratory the tubes should be removed from the thermos and arranged on the bench so that the corked ends lie away from the light source. Left like this the insects will fly towards the light and bunch together at one end of the tube. It is desirable that they be left undisturbed for at least 1 hour before any attempt is made to feed them. During this time the majority of the females cease to fly around and cluster into a tight group, often several individuals deep in places, at the lightest end of the tube.

To induce the insects to feed it is only necessary to blow gently into the tube and then invert it rapidly onto the upper arm. By this means over 90 percent of the females will usually settle down to take blood immediately. The flies can also be fed as soon as the collector has returned to the laboratory, but under these conditions only about 70 percent will take a blood meal. If it is desirable to keep the flies some time before feeding (in excess of 12 hours), they should be stored in a cool place after a strip of damp (not wet) filter paper has been inserted in the tube, together with a small ball of cotton wool containing, but not saturated with, dilute honey. Females may be kept up to 2 or 3 days before feeding in this way.

The time taken to reach full engorgement varies tremendously from about 2½ minutes to as much as 20 minutes. However, most insects are usually fully engorged within 6 minutes. It is quite apparent from the considerable distension of the abdomen when the first females are ready to leave the skin, and at this time the tube should once again be orientated so that the gorged insects will fly towards the light. Minute droplets of blood almost invariably ooze from each wound after feeding has been completed, and if the females are allowed to walk around on the skin many of them become trapped or

smeared by the drying blood. It is at this point that we see the importance of collecting only about 60 females per tube. As they reach full engorgement, and for some time afterwards, the excess water in the blood is passed to the exterior in the form of a continuous series of minute droplets, which can be seen covering the sides of the tube. If there are too many females the quantity of eliminated water becomes excessive and many females may be trapped by their wings against the sides of the tube.

For egg maturation the engorged females should be placed in 3 x 1 inch tubes containing about ½ inch of pure white sand, which has been dampened with 2 or 3 drops of water. The amount of water added at this stage is rather critical. Any excessive amount will cause condensation on the glass with drastic effects on the insects. Each tube may be stocked with up to 10 engorged females, and should then be tightly sealed and placed in a constant temperature apparatus. The temperature chosen does not matter greatly, but should not exceed 95° F. A convenient temperature is 91° F., when the flies mature eggs in approximately 30 hours and are ready to yield these after 48 hours.

Eggs are obtained by decapitation. During egg maturation many of the females burrow in the sand, and may be induced to come to the surface by light tapping of the tube. A light ethyl acetate anaesthetic should then be applied until the insects have just ceased all movement, when it is important that excess ethyl acetate vapour be blown off immediately.

As quickly as possible the flies should be transferred with a wet paint brush to small pieces of wet filter paper (approximately 1 cm. square), and orientated ventral side uppermost so that the wings adhere to the filter paper. Very shortly, twitching legs will indicate partial recovery, and at this point neat decapitation should be performed with a needle-knife. This will induce immediate oviposition in the great majority of the insects, but late starters can often be persuaded to start laying by gentle pressure on the underside of the thorax.

Treated in this way the females will deposit neat piles of eggs on the filter paper squares, which should be kept very wet until the eggs hatch. Immediately after laying the eggs are white, but soon darken to a deep brown. Kept at 91° F. they will hatch on the 4th and 5th days after laying with an average fertility of about 75 percent. Close examination will indicate when they are approaching the point of hatching, as the eye spots and black skeletal rods in the larval head capsule can easily be seen through the chorion.

*Culicoides furens.* As this species bites mainly at dawn and dusk, and is strongly affected by wind, it is somewhat more difficult to obtain than *L. bequaerti,* especially as seeing conditions are usually poor. Catching *furens* in good condition for feeding later can be an uncomfortable operation, but the area of bare skin offered can be regulated to the density of the population. It often happens that *furens* are biting in an area inhabited also by *C. barbosai.* To ensure the best chance of a good majority catch of *furens* under these circumstances it is advisable to collect only off the undersides of the legs, the area preferred by *furens,* (Kettle and Linley, 1960b).

The insects may be caught in exactly the same way as *L. bequaerti* females, and particular care should be taken to suck them very gently into the tubes for they are extremely susceptible to mechanical injury. Unlike *L. bequaerti,* the *furens* females should be fed as soon as possible after return to the laboratory, but conditions in the laboratory should be as cool as possible, preferably below 80° F. If these conditions cannot be met, very quick immersion in ice water will help.

*C. furens* females do not prefer to feed on the arm, and only very few will do so in the laboratory. Far more will take blood if the back of the thigh is offered. This poses some problem as the tube cannot simply be inverted onto the underside of the leg, unless the experimenter is lying on his back. However, if a spotlight is focused on the skin and the tube then placed on this area, most insects will fly

upwards onto the leg and a good proportion will feed. The effect is improved if the rest of the tube is blacked out with a sleeve of black paper. Unfortunately, the proportion of *furens* that will take a blood meal under these conditions is not nearly so great as with *L. bequaerti,* but over 50 percent will feed in a good batch.

Again, the engorged females should be induced to fly away from the skin by making use of the fact that they are attracted to light. During egg maturation they should be kept in tubes in the usual way except that the wet sand used with *Leptoconops* is replaced by a wide strip of filter paper dampened with a drop of water, and a source of carbohydrate is provided by a small ball of cotton wool containing diluted honey. The presence of honey is extremely important with both *furens* and *barbosai.* Almost all the *furens* females will die before eggs are matured if honey is not supplied. At 77° F. *furens* females mature eggs in about 59 hours and are ready for decapitation after 96 hours.

The procedure is exactly the same as with *L. bequaerti,* the eggs taking 4 days to hatch at 77° F., or only 2 days at 91° F., with an average fertility of about 65 percent. *C. furens* larvae seem to be dependent on the presence of a considerable quantity of water for successful eclosion. This should be borne in mind as the eggs develop towards hatching, and the filter paper squares should be kept very wet.

*Culicoides barbosai. C. barbosai* prefers to feed on the arm (Kettle and Linley, 1960c), and may be caught in exactly the same way as *furens* except that in an area where both species occur, only those insects attempting to feed on the arm should be taken. As catching *barbosai* can also be extremely uncomfortable, the following more pleasant method may be of use in certain localities. Instead of sitting out in the open, the catcher remains in his car, which is parked so that the windshield faces an area of open sky or the direction of the setting sun. If the car windows are then left open, good numbers of *barbosai* females will fly in and onto the inside of the windshield, being attracted to the light. From this position they may be gently sucked into tubes in the usual way, with a minimum of discomfort to the catcher, and in excellent condition for later experiments.

It is particularly important that *barbosai* females be subjected to a cool environment just prior to feeding. A good procedure is to place them in a thermos flask similar to that already mentioned in connection with *L. bequaerti,* and leave them for about ½ hour before offering blood. At the end of this time good numbers, (about 80–90 percent), will feed if the tube is then quickly inverted onto the upper arm.

For egg maturation, the engorged females should be kept at the same temperature as *furens* (77° F.). *Barbosai* sucks very much less blood than either *furens* or *L. bequaerti,* and it is considerably slower at maturing eggs. At 77° F. those females which have developed eggs are ready for decapitation after 144 hours (6 days), though the numbers of eggs obtained per female will be low (15 is a good number), and many individuals will not have produced eggs at all.

As with *furens,* the presence of a source of carbohydrate during egg maturation is extremely important. Without it, hardly any insects will survive the 6 days in the constant temperature apparatus. As an illustration of this point we may consider some results obtained with *barbosai* females kept at 85° F. In this series of experiments only 41 out of 75 individuals (55 percent) survived the 4-day maturation period when no honey was supplied, and of those that lived only 29 percent succeeded in maturing eggs. In contrast, when honey was available, 97 out of 109 females (89 percent) survived, and of these 54 percent succeeded in producing eggs.

The eggs take approximately 7 days to hatch at 77° F., markedly longer than *furens* eggs, and should be kept well supplied with water so that the young larvae may escape successfully. On the limited data at present available, the average fertility in batches of *barbosai* eggs obtained in this way seems to be low, (34 percent),

but the quotation of a more accurate estimate must await the accumulation of further observations.

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# ON THE HIBERNATION OF *CULEX TARSALIS* COQUILLETT, *CULISETA INORNATA* WILLISTON, AND *ANOPHELES EARLEI* VARGAS, (DIPTERA: CULICIDAE) IN ALBERTA

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INTRODUCTION. *Culex tarsalis, Culiseta inornata,* and *Anopheles earlei* are common mosquitoes in Alberta. It is generally accepted that these species overwinter in the adult stage, but little is known about the natural hibernation sites of the adults. More detailed knowledge of the winter behaviour is necessary to relate the spring density of the vectors to the ocurrence and spread of diseases in man and livestock.

MATERIALS AND METHODS. During the winter 1961–62, root cellars, abandoned mine shafts, man-made rock piles, and mammalian burrows in the irrigated areas of Alberta were searched periodically for hibernating adults. At this stage the interest was in establishing the most likely hibernating sites rather than numbers of adults.

In early September, 1962, the area south of Brooks within Alberta was surveyed for mammalian burrows. Burrows that could be visited and checked under adverse winter conditions were selected and marked. In September, 1962, traps similar to those described by Harwood and Halfhill (1960) were placed over the mammalian burrows and were then checked at least once a month. Traps containing insects were replaced by fresh traps and were brought to the laboratory where their contents were identified and recorded.

In September, 1963, 176 burrows were selected and the study area was extended northward (Fig. 1). All the burrows selected were checked periodically during the fall to determine if adult mosquitoes were entering the burrows. After the first heavy snowfall (December 3–4, 1963) the traps were set over the burrows and were left undisturbed until March 1964, at which time all the traps were checked for contents and state of repair. Damaged traps or those containing insects were replaced by fresh ones. Thereafter the traps were checked and the contents identified and recorded every two weeks. In

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