Collecting Method of the Salivary Sheath Material of Leafhoppers and Planthoppers[1]

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Collecting technique of the oral secretions of sucking insect is very important for elucidate the mechanism of feeding and virus transmission. It is well known that the saliva is ejected by an insect when it sucks the plant juice penetrating the stylet and the salivary sheath is left in plant tissue. Such salivary sheath is also formed when leafhopper probes liquid in an artificial substrate (FIFE, 1932 ; BENNET, 1934). The collecting technique of the sheath material of milkweed bug, *Oncopeltus fasciatus* (DALL) was described by MILES (1959), according to his method, the sheath material was rendered to be left by the insect in sucrose film on the microscope slide.

In this paper, some collecting methods of sheath material produced by the leafhopper and planthopper are described. The auther wish to express his hearty thanks to Prof. N. YAGI for his instructive advice.

MATERIAL AND METHOD

The following leafhoppers were used ; *Nephotettix cincticeps* UHLER, the green rice leafhopper, a vector of rice dwarf virus and rice yellow dwarf virus ; *Laodelphax striatellus* FALLÉN, the smaller brown planthopper, a vector of rice stripe virus and rice black-streaked dwarf virus.

Feeding apparatus One device is illustrated in Fig. 1, where some glass tubings (1.6 cm in diameter and 4.0 cm in length) were served as a feeding cage. Poliethylene film about 30 microns in thickness and sometimes a thin rubber or vinyl film were stuck to the end of the cage. The film was held securely in place by a rubber band and was fixed with a piece of vinyl tube of 1.8 cm in diameter and 5.0 cm in length served as the insect cage (Fig. 1,b). Ten per cent sucrose solution was poured into the glass tube corked with the other end. After the insect had been placed in the cage, the end of the cage was stuck with a cheesecloth, which was held by a rubber band. The insect was reared for 24—48 hrs. The leafhoppers and planthoppers had no difficulty in getting a foothold on the film, so that the insects could easily penetrate their mouth part through the film and suck the solution in the glass tube.

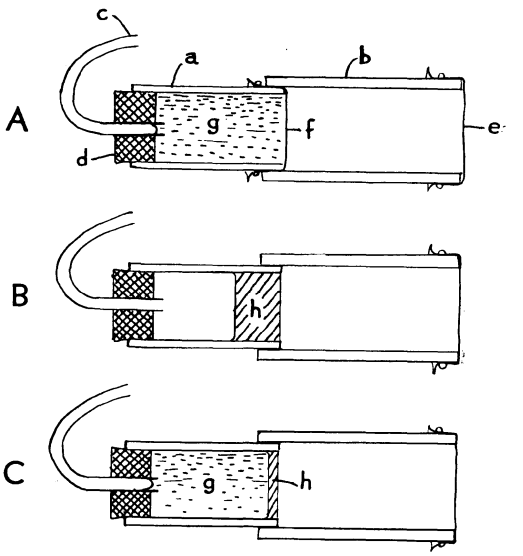

Fig. 1. Diagrams of the glass tube feeding apparatus for collecting sheath material of the leafhoppers and planthoppers : a, glass tube ; b, vinyl cage ; c, glass tube ; d, cork ; e, thin cloth ; f, poliethylene film ; g, sucrose solution ; h, sucrose contained agar.

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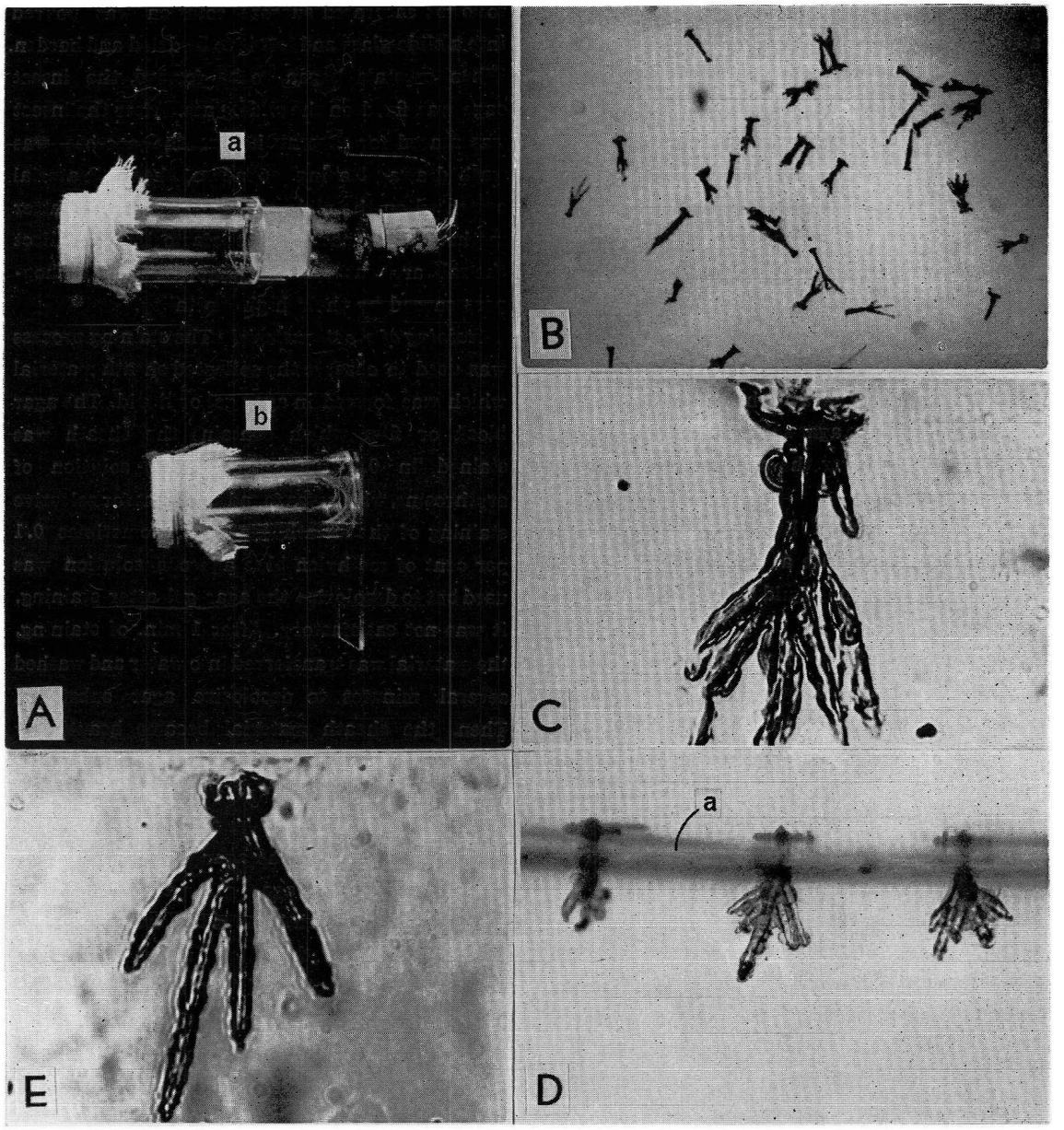

Fig. 2. Feeding apparatus for the leafhoppers and planthoppers, and their salivary sheath: A, Feeding apparatus (a) Glass tube method (b) Microscope slide method; B, Collected salivary sheath of *Nephotettix cincticeps* UHLER ×20 (secreted within agar); C, Ibid the sheath (magnifying) ×142; D, Salivary sheath deposited on the poli-ethylene film by *N. cincticeps* when the insect was fed on a sucrose solution ×100; E, Salivary sheath of *Laodelphax striatellus* FALLÉN (secreted within agar) ×300.

The agar substrate was also used in the same apparatus. First, one end of the glass tube was covered by the poli-ethylene film, which was held by a rubber band. Proper quantity of hot agar solution was poured into the glass tube, after the agar coagulated, the film was removed. After the surface of agar was dried up the insect was reared 24—48 hours in the cage as Fig. 1, B. At that time from 2 to 3 per cent of agar was suitable one, which contained 10 per cent sucrose. If the dilute agar-agar was used at that time the insects could not feed by entangling their tarsi in the agar when penetrate their mouth parts into the agar substrate. When too soft, it was better to cover the agar surface with the poli-ethylene film.

On the other hand a liquid was used jointly with the agar substrate as shown Fig. 1, C. An insect could suck the liquid media through a thin agar film.

The excretion of the insects frequently become to cause accidental drowing by their tarsi or wings, these were stuck to the wall of the cage. In order to keep the wall of the cage being clean and prevent the contamination, it was desirable to place a sheet of filter paper in the bottom of the cage. In case of necessity to get rid of entrance of fungi in these artificial media, an antiseptic substance was added.

The other more simple device was a microscope slide glass method, which was droped the sucrose agar solution in a holeslide glass to make a film of agar, and a vinyl cage (Fig. 1, b) was placed on the slide glass and fixed it with a cellophane tape, one end of the cage was covered with a cloth, which was held securely in place by a rubber band. Thus treated cage was shown in Fig. 2, A, b. The insect was reared in the cage for 25 hours. The slide glass method which was described by MILES (1959) was applied to collect salivary secretion of milkweed bug as follows; saturated sucrose solution was poured into a slide glass and left it to be dried and harden. When crystals began to be formed, the insect cage was fixed on the slide glass. After the insect was reared for several hours, the sucrose was washed away leaving mounds of sheath material cemented on the slide glass. But by the latter method it was not able satisfactorily to collect the salivary material of leafhoppers and planthoppers caused by their high mortality.

Staining of sheath material The staining process was used to observe the collected sheath material which was formed in agar gel or liquid. The agar block or film which contains the sheath was stained in 0.5 per cent aqueous solution of erythrosine, which was found excellent for selective staining of the sheath material. Sometimes 0.1 per cent of methylen blue aqueous solution was used but to decolorize the agar gel after staining, it was not satisfactory. After 1 min. of staining, the material was transferred into water and washed several minutes to decolorize agar substrate. Then, the sheath material became beautifully colored and it was observed with microscope.

Collecting of sheath material To collect the sheath material, the tubular root like sheath secreted by insect in the agar was collected by following way. The agar gel was dissolved by heating on a water bath, then the sheath material was separated and deposited in the bottom of dish, and it was collected by centrifugation (Fig. 2, B). In application of the method it is difficult to collect the watery secretion which was discharged within agar substrate or water medium.

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