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Collecting Method and Preliminary Analysis of the Soluble Salivary Secretions of the Planthoppers[1]

The analysis of salivary secretions of phytophagous Hemiptera is undoubtedly an important subject for the aetiological studies on the phytotoxemia caused by the feeding of those insects. Although studies have been made on the coagulable '*sheath material*' (SMITH, 1933; MILES, 1960; SŌGAWA, 1967) and the digestive enzymes in the salivary glands (SAXENA, 1954; NUORTEVA, 1954; FEIR and BECK, 1961), the information available for the components of the soluble salivary secretions is still very scanty, excepting several references to amino acids (KLOFT, 1960; SCHALLER, 1961) Recently it has been demonstrated that various compounds are transported from heamolymph to the saliva (MILES, 1967). This evidence suggests the complexity of the components of the saliva.

The present note refers to the collecting method and preliminary analysis of the soluble salivary secretions of the planthoppers, *Nilaparvata lugens* STÅL and *Laodelphax striatellus* FALLÉN.

METHOD FOR COLLECTION OF SALIVA

The apparatus used to collect the saliva was illustrated in Figs. 1 and 2. A sheet of filter paper (Toyo filter pater No. 51) which is constantly moistened with distilled water flowing desendingly is exposed to numerous probing of the planthoppers. The saliva discharged is carried downward and eluted from the lower end of the filter paper by distilled water. The filter paper which is allowed to probe is 40 cm by 10 cm in size, and set 45 degrees to prevent contamination of excreta. Also its outer surface is covered by a glass plate in order to prevent drying. Usually two

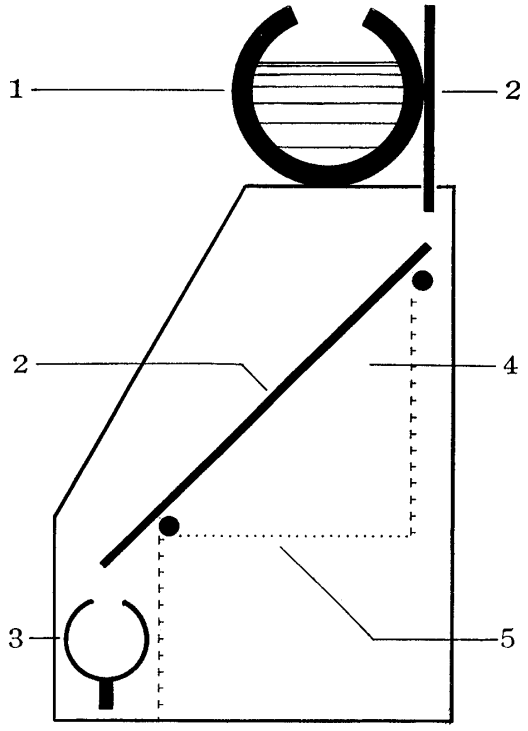

Fig. 1. Structure of the apparatus for collection of the soluble salivary secretions of the planthoppers. 1. plastic vessels of distilled water; 2. glass plates along which filter paper is set; 3. glass vessel for collecting the solution; 4. chamber in which the planthoppers are confined; 5. fine wire gauze.

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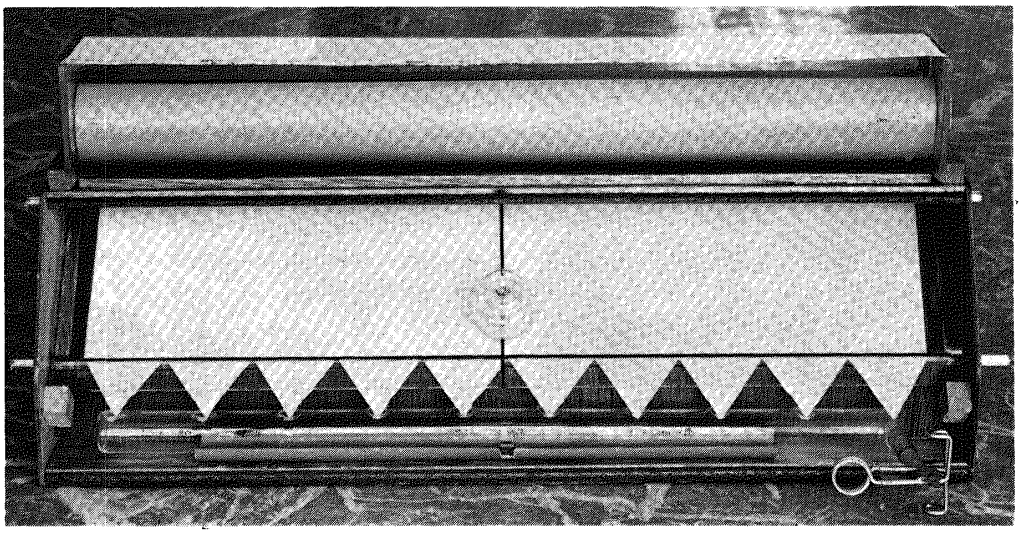

Fig. 2 Front view of the collecting apparatus (1/4).

to three thousands of the planthopper adults are introduced into the apparatus and allowed to probe for 3 to 4 days.

PRELIMINARY ANALYSIS OF SOLUBLE SALIVARY SECRETIONS

Lipids: The solution collected was condensed to a small volume with rotary vacuum evaporator at 50°C. Lipids were extracted according to the method of FOLCH et al. (1957). The extract was chromatographed unidimentionally on a thin-layer of silica gel G using hexane-ether (9 : 1 v/v) as solvent system. Lipids were detected by 80 % sulfuric acid saturated with potassium dichromate. Typical chromatograms obtained were shown in Fig. 3. The occurrence of hydrocarbon, triglyceride and phospholipid was indicated.

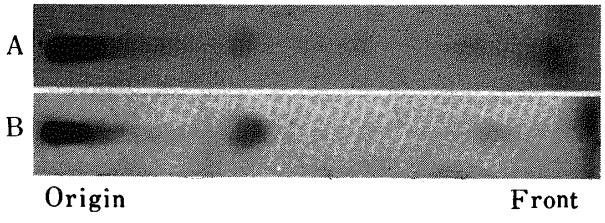

Fig. 3. Thin-layer chromatograms of lipids in the salivary secretions of *N. lugens* (A) and *L. striatellus* (B).

Protein: When the collected solution was condensed and treated with ethanol, a small amount of precipitate occurred. It was insoluble in 80 % ethanol but soluble in distilled water in colloidal. Several amino adids and a few amino sugars were detected from the hydrolisate of the precipitate, indicating a mucoprotein.

Amino acids: Free amino acids were extracted with 80 % ethanol after removing lipids, separated by means of unidimentional paper chromatography using butanol-acetic acid-water (4 : 1 : 2 v/v) as solvent system, and detected with 0.025 % ninhydrin in acetone. The presence of alanine, arginine, valine, leucine (and/or isoleucine), etc. was indicated by comparing with the chromatogram of authentic amino acids as shown in Fig. 4.

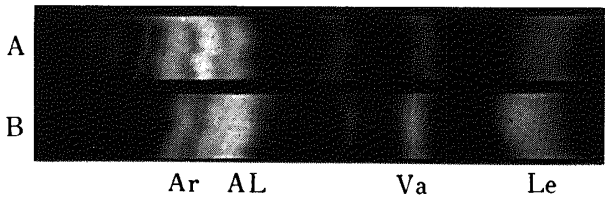

Fig. 4 Paper chromatograms of free amino acids in the salivary secretions of *N. lugens* (A) and *L. striatellus* (B).

EHRLICH-*Positive substances* : After extraction of lipids, soluble compounds in ether-methanol (4 : 1 v/v) were extracted and submitted to paper chromatography using butanol-aceticacid-water, (4 : 1 : 2 v/v) as solvent system. The compounds were detected with EHRLICH's reagent and glutaconic aldehyde reagent prepared by adding sodium hydroxide to 1 % alcoholic solution of 4-pyridylpyridinium dichloride. Usually

three spots were recognized on the chromatogram as shown in Fig. 5. The two spots at Rf 1.5-2.0 and 4.5-5.0 gave a yellow EHRLICH's reaction and produced an orange color with the glutaconic aldehyde reagent. Another one tailing near Rf 8.5 gave a purplish color with EHRLICH's reagent.

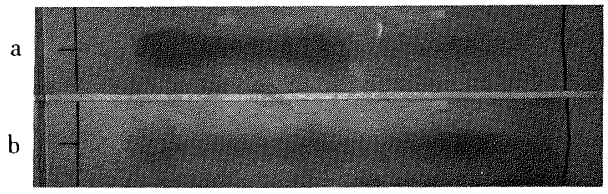

Fig. 5. Paper chromatograms of EHRLICHpositive substance in the salivary secretions of *N. lugens*. Similar chromatograms were obtained with the secretions of *L. striatellus*. (a) sprayed with glutaconic aldehyde reagent, (b) with EHRLICH's reagent.

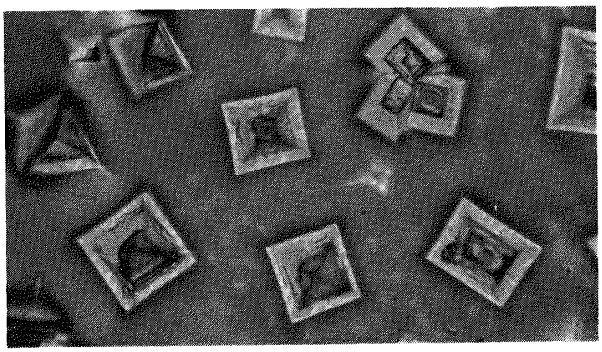

Fig. 6. Crystals of an inorganic salt in the salivary secretions of *N. lugens*. Similar those were also recognized in the secretions of *L. striatellus*.

Inorganic salt: Crystls shown in Fig. 6 appeared when the condensate of proteinized solution was kept in refrigerator. They were insoluble in organic solvents, slightly soluble in distilled water and readily soluble in diluted hydrochloric acid; and were indicated to be an inorganic substance by burning test. The qualitative tests showed the positive reactions for calcium and phosphorous ion. It is therefore likely that the crystals are calcium phosphate.

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