not as indicating intention to make a study of the subject.

The conditions were about as follows: Two grams of air-dry powdered soil with 200 cc of distilled water were placed in 500 cc wide-mouth bottles closed by rubber stoppers. After shaking the mixture for a definite time, it was filtered on ashless filter-paper on a Buchner funnel by the aid of suction. The first of the filtrate, which was turbid, was returned to the filter until it came through clear. In the clear solution, PO_4 was determined by the molybdenum blue method.

Two quite different shaking machines were employed. One had a reciprocal motion with a stroke of about two inches at the rate of 120 per minute. In this the bottles lay on their sides so placed that the motion was lengthwise of the bottles. Since the bottles were less than half full, their contents were rather violently agitated. The longer the time of shaking the greater was the amount of PO_4 dissolved from sandy soils. With clay soils time did not make so much difference.

In the other shaking machine, the bottles were placed with their longer axes perpendicular to the axis of the rotating holder in such manner that they were turned end over end at the rate of four revolutions per minute, which scarcely did more than keep the contents of the bottles mixed by very gentle agitation. After it was found that the method of shaking made much difference in the amount of phosphate dissolved, the position of the bottles in the end-over-end shaker was changed so that their long axes were parallel to the axis of the machine. In this way, the bottles were turned over by a sort of rolling motion which kept the contents in motion without violent agitation. The rotation was so gentle that an ordinary filter-paper placed in the soil suspension was not torn after two hours of agitation. In the reciprocal shaker, the filter-paper was disintegrated to pulp in a few minutes.

Results recorded in the appended table seem to indicate that about one hour's agitation in the end-over-end shaker is long enough for clay soils. With even this gentle motion, the PO_4 dissolved from soil 30, a fine sandy loam, increased with the length of time shaken, so that the length of time for shaking a sandy soil has been arbitrarily set at one hour, with the knowledge that slight changes in the conditions may cause considerable difference in the results. It appears probable that discordant results in the analysis of soils for various constituents have frequently been caused by differences in the method of mixing or agitating the suspension before filtering off the solution.

In this connection, it is of interest to note that workers in physical analysis of soils observe that the amount of colloidal matter extracted from some soils is increased by longer time of agitation of the suspension before making the separation.

EFFECT OF METHOD OF SHAKING ON PO_4 EXTRACTED FROM SOILS BY SHAKING WITH WATER

Kind of shaker	Time shaken	Soil numbers: Silty clays, No.'s 1c	38	65	Sandy soils, No.'s 30	53	68
		p.p.m. PO_4 in air-dry soils					
Reciprocal	1 hour	50	63	316	234	74	37
"	2 "				274		
End over end		49	49	316	100	37	30
Rotary	1 min.	21					
"	1 hour	42					
"	2 "	45					
"	½ "	40			58		
"	1 "	44			68		
"	2 "	43			82		
Shaken by hand once every five minutes for one hour, then filtered:							
Very gentle shaking		57					
Violent shaking		65					

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A METHOD OF ARTIFICIALLY FEEDING THE SUGAR-BEET LEAFHOPPER

CARTER[1, 2] devised an apparatus for the purpose of artificially feeding the sugar-beet leafhopper, *Eutettix tenellus* (Baker). He pointed out that this device is also suitable for studies on the properties of the curly-top virus and for nutritional studies with sucking insects.

For certain biochemical investigations on the curly-top problem, Carter's apparatus was found to be unsuitable. It became necessary therefore to devise a method whereby the sugar-beet leafhopper and other closely related species could be fed artificially on very small amounts of solutions of known composition.

Pieces of glass tubing 1.5 cm in diameter and 2 cm in length served as the cage. One end of the cage was covered with cheesecloth, which was held securely in place by a rubber band. A section of paraffin ribbon, cut 60 microns in thickness with a microtome, was stuck to the other end of the cage after the leafhopper had been placed inside. The assembled cage is shown in Fig. 1. The animal mesentery membranes or the baudruche capping skins used by Carter were

[1] *Jour. Agr. Res.*, 34: 449–451, 1927.
[2] *Phytopath.*, 18: 246–247, 1928.

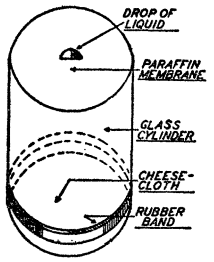

FIG. 1. Feeding device for the sugar-beet leafhopper. The insect caged in the glass ring punctures the paraffin membrane and feeds on the drop of solution.

found to be very unsatisfactory for pH determinations. When this type of membrane is wetted by liquids, adsorption of ions takes place and a serious error is introduced into the determinations. The paraffin membranes being chemically inert and easy to prepare and handle were found to be well adapted for the studies carried out.

The leafhoppers have no difficulty in getting a foothold on the paraffin membrane. There was usually a delay in finding the drop of liquid placed on the membrane if no assistance was given the leafhopper. To expedite matters, the practice was followed of waiting until the leafhopper began to puncture the membrane and then placing a drop of the liquid directly over the leafhopper. In this way it was possible to induce hungry leafhoppers to feed almost at will. The drop placed on the membrane can be protected from air and evaporation by an air-tight compartment made of the same size glass tubing. The drop can even be subjected to different gases by using such a covering chamber into which side tubes are sealed.

To study the pH of saliva injected into the drop by a leafhopper, one was allowed to feed on a drop (.01 cc) of slightly buffered 5 per cent. sugar solution. The pH determinations made on a large number of leafhoppers show that the material injected into the drop of liquid by the leafhopper is very alkaline. This was confirmed by allowing leafhoppers to feed on a drop of brom thymol blue. The drop of brom thymol blue (made acid) would turn from a lemon yellow to a deep green and in some instances to a blue color within three minutes after the leafhopper had started to feed.

In the early part of the work it was observed that some leafhoppers, while feeding, ejected a substance which very quickly coagulated around their mouth parts. To study this process another cage was designed so that it could be mounted on a slide and placed under the microscope, Fig. 2. Narrow strips were cut from the end of a micro-slide approximately 1.5 mm in thickness. Small compartments were made by sealing two such pieces parallel with each other

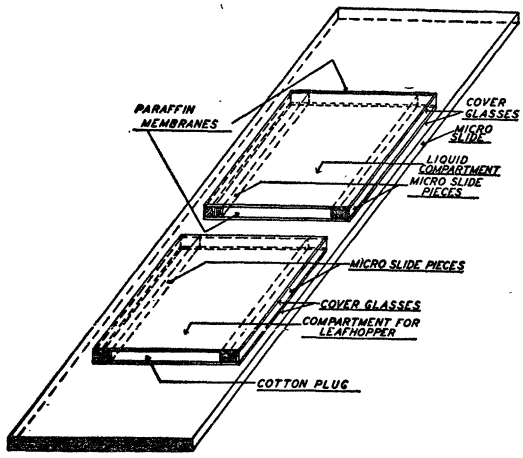

FIG. 2. Feeding device arranged to permit observation under the microscope of the sugar-beet leafhopper feeding upon the test solution.

between two square cover-glasses. Either paraffin or duco-cement may be used to seal the pieces together. A paraffin membrane 120 microns in thickness was placed across the end of one of these compartments and gently pressed in position. The liquid, on which the leafhopper was to feed, was placed in this compartment, which was then sealed with another paraffin membrane. The distortion due to the highly curved surfaces of a drop was entirely eliminated by such a compartment. The compartment containing the liquid was sealed to a glass slide with a strip of paraffin ribbon. The open end of another compartment was pushed against the membrane on the liquid compartment. This formed a cage for the leafhopper having the paraffin membrane at one end and a cotton plug at the other. With this arrangement the leafhopper was forced to feed on its side which placed its mouth parts (when through the membrane) at right angles to the line of vision through the microscope. With the leafhopper feeding in this position the mouth parts can easily be seen in action under low or high power. It is an easy matter to see the coagulable material ejected at the tip of the setae bundle.

These devices open up a wide range of interesting possibilities for study on the properties of the curly-top virus, the mechanics of feeding and the chemical nature of the leafhopper saliva. Detailed studies on the pH of the saliva of the sugar-beet leafhopper, which have been carried on as part of a study of the chemical nature of disease resistance in the case of the curly-top disease, will be presented later.

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