of prime importance in the development of fungi on root surfaces.

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ENTOMOLOGY

Persistence of *Erwinia amylovora* in the Apple Aphid (*Aphis pomi* DeGeer), a Probable Vector

Insects of the family Aphidae and particularly the apple aphid, *Aphis pomi* DeGeer, have long been suspect as significant vectors of the phytobacterial pathogen *Erwinia amylovora* (Burrill) Winslow *et al.*, 1920. That these insects could artificially transmit the fireblight bacterium and establish progressive infections in apple and pear tissue was demonstrated by Stewart[1], Stewart and Leonard[2], and Merrill[3].

It was, however, not known how soon after feeding, or for how long, the pathogen could be detected within the insect. These questions remained unanswered because feeding the apple aphid *in vitro* was, until recently, an all but impossible task.

Recently in our laboratory, and independently by Mitler and Dadd[4] and Strong[5], a system has been developed by which aphids can be successfully fed a synthetic substrate through an artificial membrane. Our simple glass feeding chamber is shown in Fig. 1 and is essentially two 3-in. pieces of glass tubing 4 mm in diameter, separated by a thinly stretched membrane of 'Parafilm-M' (Marathon Co., Menasha, Wisconsin). The Parafilm was secured with a rubber band and one of the glass tubes was drawn slightly smaller in diameter than the other so that the smaller one could be inserted into the larger.

The aphids were placed on one side of the membrane and a droplet of sap expressed from young shoots, containing a suspension of the fireblight bacterium, was placed on the other side. Active feeding by the aphid through the membrane is shown in Fig. 2. During the feeding period, the tubes were inverted so that the insect adopted approximately its normal feeding attitude on the under side of the apple or pear leaf. Furthermore, feeding in this manner prevented the dropping of ingested bacteria to the membrane surface in aphid excrement, and reduced the likelihood of contaminating the external surface of the insect.

To obviate the necessity for aseptic procedures, a virulent strain of *E. amylovora* was developed that was resistant to 1,000 μg/ml. streptomycin. The use of this genetically marked mutant permitted ready separation of *E. amylovora* from the endogenous bacterial flora of the aphid as well as atmospheric contaminants.

In replicate experiments, ten single aphids were fed the apple juice bacteria mixture for intervals of active feeding ranging from 5 min to 2 h. In another experiment, three groups of insects fed for 2 h were placed on vigorously-growing apple shoots. The insects were subsequently removed from their natural host at intervals, and macerated in streptomycin-containing broth which was plated on streptomycin-containing agar.

The results from these experiments appear in Tables 1 and 2. They reveal that the pathogen could be found in the bodies of the insects after as short a feeding period as 5 min. Moreover, the bacteria persisted in the aphid for at least 72 h, the duration of the longest experiment.

Table 1. Time Interval after Feeding when Bacteria could be recovered from the Insects

Time (min)	5	15	30	60	120
No. of positives (out of 10)	2	5	3	4	6

Table 2. Persistence of Bacteria in the Insect

Time (h)	24	48	72
No. of positives (out of 10)	4	4	2

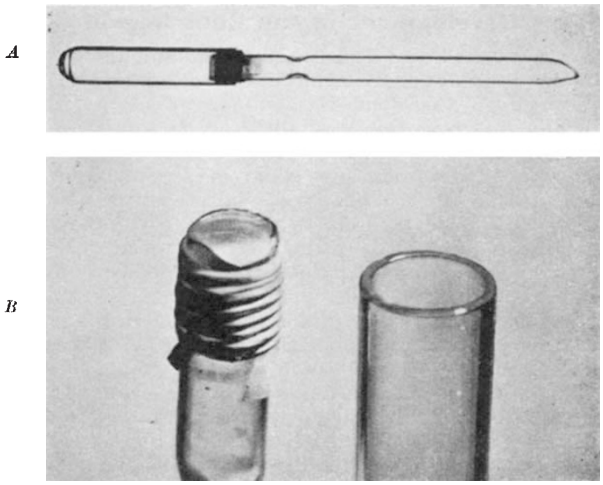

Fig. 1. Insect feeding chamber. *A*, assembled; *B*, unassembled

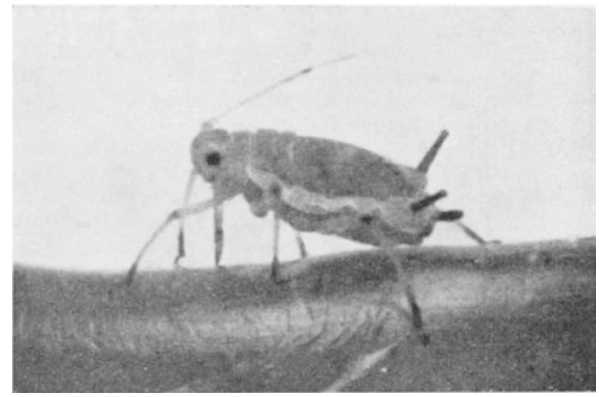

Fig. 2. Aphid actively feeding through a 'Parafilm-M' membrane

To determine whether or not contamination of the external surface of the membrane had occurred as a result of feeding, this surface, on which the aphids had rested, was swabbed with cotton and promptly streaked over the streptomycin-containing agar. In no instance was the pathogen recovered from the surface of the membrane. Hence, it would appear that our isolation of *E. amylovora* from *Aphis pomi* was, in fact, bacteria that had been ingested by the insect.

Experiments are now in progress to determine the distribution of the pathogen in the internal organs of the aphids and the approximate number of bacterial cells required to establish a progressive infection by *Aphis pomi* in host tissue.

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