

# Proceedings of the Iowa Academy of Science

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Volume 70 | Annual Issue

Article 80

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1963

## Survival of *Empoasca labae* (Harris) (Cicadellidae, Homoptera) on Synthetic Media

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### Recommended Citation

Dahlman, Douglas L. (1963) "Survival of *Empoasca labae* (Harris) (Cicadellidae, Homoptera) on Synthetic Media," *Proceedings of the Iowa Academy of Science*: Vol. 70: No. 1 , Article 80.  
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experience of the trials in the snails. It is believed that the amount of learning, if any, that occurred was insufficient to be measured in any way. It is not believed to be a major factor, and does not noticeably affect the snail's reaction time.

## Survival of *Empoasca fabae* (Harris) (Cicadellidae, Homoptera) on Synthetic Media<sup>1</sup>

DOUGLAS L. DAHLMAN<sup>2</sup>

*Abstract.* Nymphs of the potato leafhopper were caged in small clear plastic snap boxes for individual survival studies. All experiments were conducted at 85°F in a darkened incubator, minimizing responses to light and varying temperature. The leafhopper nymphs were sustained on an agar gel matrix containing sugars and amino acids. Relative nutritional adequacy was evaluated by the time required to reach 50% survival. The lowest mortality rate occurred on a 7% sucrose-2% agar medium. Preferential utilization of the glucose fraction of sucrose was indicated. A medium composed of agar, sucrose, and the 10 amino acids essential for human and rat nutrition tripled the 50% survival time compared with agar controls.

Feeding by potato leafhopper, *Empoasca fabae* (Harris), induces extensive foliage injury (hopperburn) in many tuber bearing *Solanum* species. *Solanum tuberosum*, the commercial potato, is especially susceptible (Ball, 1919). It has been postulated that toxic substances injected into leaf tissue during leafhopper feeding induce hopperburn injury (Fenton and Resler, 1922; Eyer, 1922; Granovsky, 1926). Nymphal stages produce the greatest amount of injury (Fenton, 1921).

Although knowledge of the nutritional requirements of insects is important to understanding insect-host relationships, to developing economic controls, and to maintaining these animals in the laboratory, the basic nutritional requirements of the potato leafhopper have not been reported in the literature.

Nutritional requirements have been completely defined for only a small number of insect species. Partial requirements have been defined for a larger number (Albritton, 1954). In most cases, investigators presented solid or semisolid diets to chewing insects. Criteria used for evaluation of relative nutritional adequacy were survival, growth and development, and adult fertility (Trager, 1953). More recently, the nutrition of

<sup>1</sup> Journal Paper No. J-4592 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project 1434. This study was supported in part by a research grant G-10923 from the National Science Foundation.

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insects with piercing-sucking mouthparts has been studied (Scheel, Beck, and Medler; 1956). It is difficult to present a synthetic medium to such insects because it must be liquid or semisolid.

Many different natural and synthetic membranes have been employed to separate the insects from gross bodily contact with the liquid medium. Usable membranes must be thin enough to allow the insect's mouthparts to penetrate, but must be sufficiently firm to retain the fluid medium and to endure multiple probing by the insect. Scheel, *et al.* (1956) reviewed the membrane-technique methods that have been tried during the past 30 years.

An alternative to the membrane-feeding technique is to impregnate a nutritionally inert matrix with a nutrient solution. The insect may feed from the matrix without becoming trapped in the nutrient-bearing liquid. Agar and gelatin are useful alternatives to membranes but may not be nutritionally inert.

The objective of the experiment reported in this paper was to devise a medium which would sustain the potato leafhopper in the laboratory during extended periods of observation.

#### MATERIALS AND METHODS

An infestation of leafhoppers was maintained on broad bean, *Vicia faba* L., in the Iowa State University insectary greenhouse.

Since, in preliminary experiments, adult leafhoppers survived for three to four days on 2% agar (w/v) without added nutrients, agar was used as a matrix to which nutrients were added to study their influence on duration of nymphal and adult survival.

Clear plastic snap boxes 1" x 1" x 1/2" served as cages for the leafhoppers during the test periods. About 2½ ml of hot 2% agar were poured into each half of the box. As soon as the agar gelled, 1" squares of filter paper were placed to completely cover both agar surfaces. This prevented leafhoppers from becoming caught on the agar. Within the closed cage, there were two surfaces upon which feeding could occur — one facing upward, and the other, inverted.

An aspirator was used to collect fourth and fifth instar leafhopper nymphs from caged broad bean plants. The nymphs, briefly anesthetized with CO<sub>2</sub>, were placed in the plastic test cages, one nymph per cage. Injured nymphs, detectable under a dissecting microscope, were replaced with uninjured specimens.

All tests were conducted at 85°F in a darkened incubator, minimizing responses to light and varying temperature. Twenty

cages of each test diet or control treatment were used. During each experiment, leafhoppers were transferred to freshly prepared cages on alternate days to minimize contamination from bacteria and mold. For each leafhopper, the approximate time of molting was recorded. Frequent checks for mortality permitted estimation of the approximate number of hours that individual leafhoppers survived.

### RESULTS

The effect upon leafhopper survival of sucrose incorporated into the agar matrix was tested. Seven experiments were completed, each using 20 leafhoppers on 2% agar control and 20 leafhoppers on 1% sucrose-2% agar. In a representative experiment, 50% of the leafhoppers remained alive after 1.8 days on the 2% agar control. The 50% survival time was extended to 3.2 days on agar containing 1% sucrose (Fig. 1).

Since leafhoppers on the 1% sucrose-2% agar medium lived nearly twice as long as those on the control, an experiment was designed to measure the effects on survival of glucose and fructose, the monosaccharide components of sucrose. Equimolar quantities (0.029 M) of sucrose, glucose, and fructose

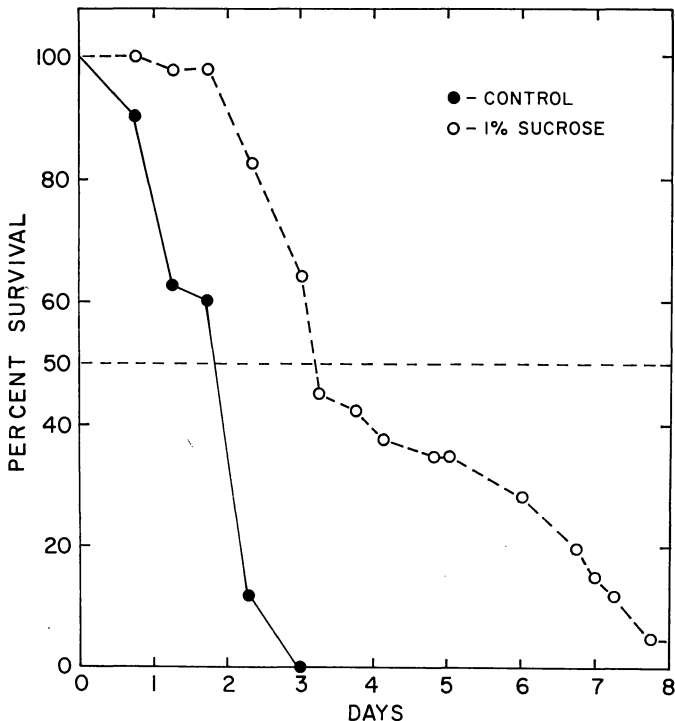


Figure 1. Potato leafhopper survival on 2% agar control and 1% sucrose-2% agar.

were used in separately prepared media, The 50% survival point occurred after 2.3 days on the control, after 2.1 days on fructose, after 3.0 days on glucose, and after 3.6 days on sucrose (Fig.2) Repetition of the experiment gave similar results.

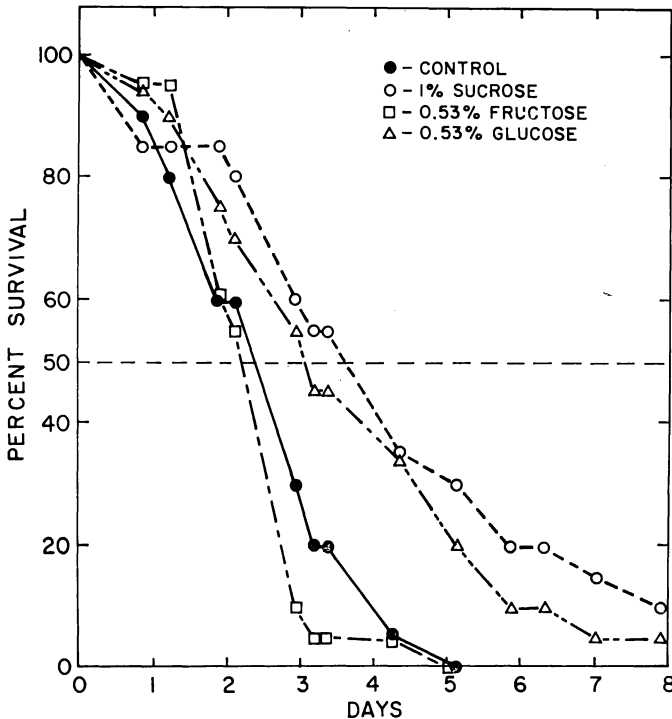


Figure 2. Potato leafhopper survival on 2% agar control and equimolar concentrations of sucrose, fructose, and glucose.

An experiment was designed to test the influence of a mixture of amino acids and vitamins on leafhopper survival. A modification was used of the synthetic medium developed by Mittler and Dadd (1962) for the green peach aphid, *Myzus persicae* (Sulzer). That diet specified 20 different amino acids, 10 different vitamins, sucrose, cholesterol,  $MgCl_2 \cdot 6H_2O$ , and  $K_3PO_4$ . The constituents were incorporated into 2% agar, but 1% sucrose was used instead of 18%. It was found that 50% survival was attained in less time on the modified aphid medium than on the agar control.

However, when only the 10 amino acids essential for human and rat nutrition (Gilmour, 1961) and 1% sucrose were incorporated into a 2% agar matrix, the time required to reduce survival to 50% was nearly four times that observed on the agar control (Fig. 3). The amino acid mixture consisted of

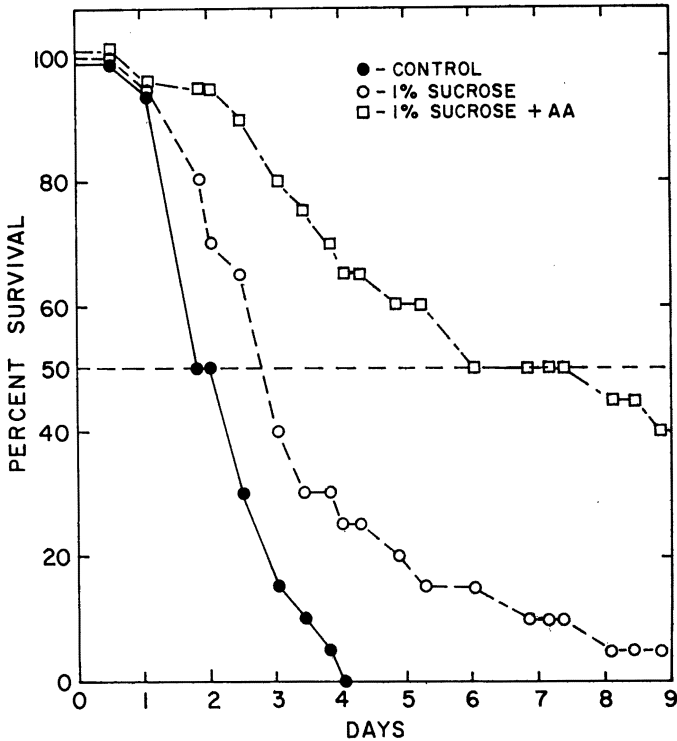


Figure 3. Potato leafhopper survival on 2% agar control, 1% sucrose-2% agar, and 1% sucrose-2% agar plus 10 "essential" amino acids.

27 mg arginine, 4 mg histidine, 3 mg isoleucine, 4 mg leucine, 12 mg lysine, 1 mg methionine, 1 mg phenylalanine, 14 mg threonine, 4 mg tryptophan, and 4 mg valine added to 100 ml of 1% sucrose-2% agar.

The influence of sucrose concentrations of 1, 3, 5, 7, and 10% (w/v) on leafhopper survival was tested. The times in days required on these media to reduce survival to 50% were: 1.6 for agar control, 4.5 for 1% sucrose, 5.7 for 3%, 5.7 for 5%, 8.4 for 7%, and 4.1 for 10% sucrose. These data suggested an optimal sucrose concentration near 7%.

#### DISCUSSION

Highest leafhopper mortality occurred during molting of fourth and fifth instar nymphs, usually within the first three days following placement of nymphs on the synthetic medium. This was particularly evident on controls. The early mortality was reduced on diets that extended the period of survival (Figs. 3 and 4), and reduced early mortality may be attributable to improved nutrition.

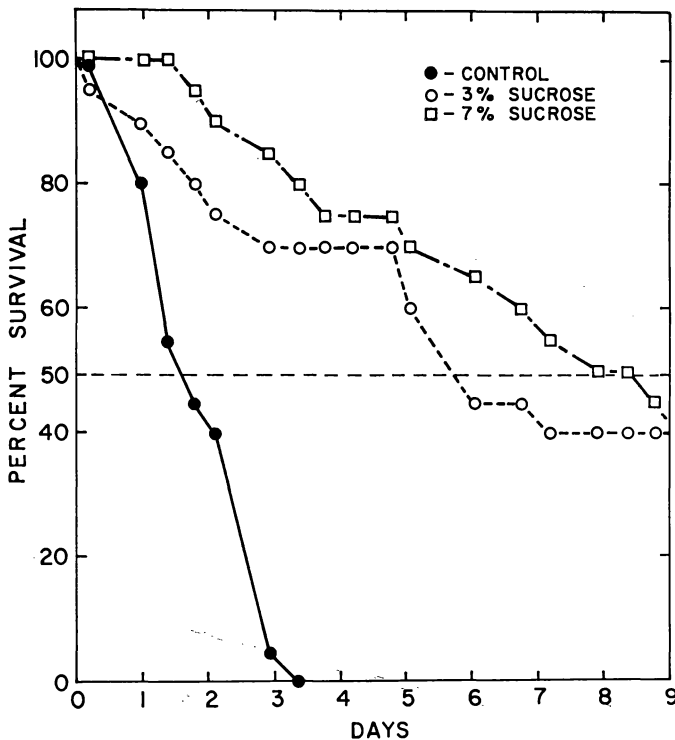


Figure 4. Potato leafhopper survival on 2% agar control, 3% sucrose-2% agar, and 7% sucrose-2% agar.

The lowest potato leafhopper mortality rate occurred on a 7% sucrose-2% agar medium. Survival on 0.029 M glucose was nearly the same as that on equimolar sucrose and greater than that on equimolar fructose, indicating preferential utilization of glucose.

The 10 "essential" amino acids added to a 1% sucrose-2% agar medium more than tripled the 50% survival time. Further tests to identify those amino acids essential to normal potato leafhopper development would be of interest.

The practicability of an agar gel-based medium for sustaining the potato leafhopper during nutritional and behavioral studies was indicated by the results of these experiments.

ACKNOWLEDGEMENTS

Sincere appreciation is expressed to Drs. E. T. Hibbs and W. H. Orgell for their advice and constructive criticism in the preparation of this manuscript.

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## Acarine Fauna of Bird Nests<sup>1</sup>

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*Abstract.* In summer, 1961, 10 nests, representing seven species of passeriform birds, were collected and processed in Berlese funnels to obtain the mites present. Representatives of 20 different families or superfamilies were identified in addition to five groups of larvae and nymphs which could not be identified to family. The families Eremaeidae and Dermanyssidae were most widely represented, and dermanyssids were most numerous.

Mite populations are believed to become established in nests by (1) mites being brought into the nest by way of nest materials, (2) mites being brought into the nest on the bird itself, and (3) mites in their wanderings accidentally encountering the nests.

Investigation and analysis of mite populations in bird nests have received but scant attention, and most of this has resulted incidentally from studies conducted for other purposes. Cameron (1938) presented information on the occurrence of the northern fowl mite in nests of several species of passerine birds. The attention of additional workers has been directed to this species as well as to other mites concerning their possible role in the epidemiology of encephalomyelitic pathogens. A different approach, involving nests as sources or reservoirs for contamination of foodstuffs and other stored products by mites, has been used by Kemper (1938) as well as by others. Of the few investigations directed toward understanding the commensal and parasitic relationships in a nest, the more outstanding contributions are

<sup>1</sup> A research grant awarded by the National Science Foundation to promote undergraduate research made this study possible.

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