Evaluation of Artificial Diets for Rearing *Aphis glycines* (Hemiptera: Aphididae)

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ABSTRACT Artificial aphid diets have been previously developed for the pea aphid, *Acyrthosiphon pisum* (Harris), and the green peach aphid, *Myzus persicae* (Sulzer). The ability to rear aphids on an artificial diet allows for selectively adding or subtracting compounds from an aphid's food source to determine the effect on fecundity and longevity. Five diets previously developed for the green peach aphid and the pea aphid were tested for their suitability for rearing soybean aphid, *Aphis glycines* Matsumura. The best diet, originally developed for the green peach aphid and based on the amino acid profile of young potato plants, allowed 12 generations of soybean aphids to develop. For all diets tested, aphid fecundity, and longevity were greatly reduced in comparison with aphids reared on soybean, *Glycine max* (L.) Merr., plants or on detached soybean leaves. In addition, mean developmental time was significantly longer for aphids reared on artificial diets.

KEY WORDS soybean aphid, *Aphis glycines*, artificial diet, nutrition

The soybean aphid, *Aphis glycines* Matsumura, is an economically important pest that feeds solely on the phloem of mostly soybean, *Glycine max* (L.) Merr., and buckthorn (*Rhamnus* L.) plants. Phloem is high in sugars and nitrogen in the form of amino acids, and it is often lacking feeding deterrents or toxins (Douglas 2006). Although it is a good source of carbohydrates, phloem has a low concentration of most of the essential amino acids; therefore, many aphid species, including the soybean aphid, have associations with symbiotic bacteria known to contribute amino acids that are lacking in the aphid's diet (Febvay et al. 1999). Ten amino acids are known to be essential to all insect species; however, symbionts in the green peach aphid, *Myzus persicae* (Sulzer) contribute seven of the ten essential amino acids. If the symbionts are killed with antibiotics, the aphid requires all 10 essential amino acids in its diet to survive (Dadd and Krieger 1968).

The artificial diet requirements of the green peach aphid have been studied for many years. The first aphid artificial diet was developed by Dadd and Mittler (1965), which allowed researchers to rear larval green peach aphids to the adult stage on a liquid media composed of sucrose, amino acids, vitamins, and water. Although necessary for insect development, sterols were not required in the aphids' diets (in fact, diets containing cholesterol showed a decrease in aphid development), and they were presumed to be contributed by symbionts.

The dietary requirements of the pea aphid, *Acyrthosiphon pisum* (Harris) also have been studied. Artificial diets developed for the pea aphid established that in addition to amino acids, sucrose and vitamins, trace minerals are also necessary for aphid development and reproduction. Diets containing iron, copper, manganese, and zinc as chloride salts were found to increase the rate of reproduction and the adult mass in the pea aphid (Akey and Beck 1972). Using glutamine labeled with heavy nitrogen, Sasaki et al. (1991) established that arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and valine are all produced by the aphids' symbionts, and they are therefore not essential in an artificial diet.

There are several benefits to rearing aphids on artificial diets. Aphids are traditionally reared on their host plants, but the exact composition of the plant's phloem is rarely known, and it can vary based on temperature, stress, or the life stage of the plant. Artificial diets allow the researcher to rear aphids on a chemically defined diet that remains consistent regardless of environmental conditions. In addition to being chemically defined, artificial diets allow the easy introduction or removal of chemical compounds from an aphid's diet. Artificial diet studies often require less space, because aphids are reared in petri dishes instead of on plants.

Materials and Methods

Aphids and Diet Chamber Construction. A soybean aphid clone from Urbana, IL, that was initially collected and maintained by Curt Hill (Department of Crop Sciences, University of Illinois, Urbana, IL) was used in all experiments. The clone was established from a single first instar reared continuously on Williams 82 soybean plants in plant growth chambers, with each population maintained in a separate growth

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chamber. The growth chambers were kept at 22°C, 70% RH, and continuous 300 μmol m^{-2} s^{-1} photosynthetically available radiation. All experiments were conducted with nymphs <6 h old.

Diet chambers were constructed with two sizes of petri dishes: a 60- by 15-mm small dish (Falcon 351007, Becton Dickinson Labware, Franklin Lakes, NJ), and a 100- by 15-mm large dish (Fisher, Hampton, NH). A piece of white 90-mm filter paper (Whatman, Mobile, AL) was placed in the bottom of the large petri dish to allow better visualization of the aphids. One 7- by 7-cm sheet of Parafilm M (SPI Supplies, West Chester, PA) was stretched across the opening of the top of a small petri dish, and 2 ml of artificial diet was placed on top of the Parafilm M layer. A second sheet of Parafilm M was then stretched over the first sheet, creating a diet sachet on top of the small petri dish. The small petri dish, covered with the diet sachet, was then placed inside the larger petri dish (Fig. 1). Aphids were then placed on top of the diet sachet by using a paintbrush, and covered with the lid of the large petri dish.

Initial Feeding Studies. To determine the ability of soybean aphids to probe and feed through a Parafilm M membrane, an initial feeding study was conducted consisting of four different treatments. The positive control treatment consisted of aphids feeding on detached Williams 82 soybean leaves placed in a petri dish containing moistened filter paper. The negative control treatment consisted of a diet chamber with water in the diet sachet, and it was included to test the development and mortality of soybean aphids in the absence of a food source. The first experimental treatment consisted of an aphid diet chamber with a detached Williams 82 soybean leaf sandwiched in between two layers of Parafilm M, with the underside of the leaf available for the aphids to feed on. A final experimental treatment consisted of a diet sachet filled with 2 ml of 10 g of Williams 82 soybean leaves ground in 3 ml of water by using a leaf grinder (manufactured by Erich Pollähne, Wennigsen, Germany). Food sources for all experimental treatments were changed two times a week throughout the study.

Eight replications, each consisting of ten aphids on a diet sachet or detached leaf, were performed for each experimental treatment. For each replication, ten aphid nymphs were transferred onto a diet sachet or detached leaf by sing a sable-hair brush. Mean developmental time from nymph to adult, fecundity, and longevity were measured in days for all experiments. Fecundity was measured as the number of offspring that an aphid produced throughout the course of its adult life, and longevity was calculated as the total number of days the aphid lived. Aphid nymphs were removed after they were counted to eliminate overcrowding.

Artificial Diet Studies. Five different previously described diets, differing only in amino acid content, were used (Table 1). Diet A0 (Febvay et al. 1988) was based on the total amino acid profile of the pea aphid. Diet A1 (Febvay et al. 1988) was a variation of diet A0 with a reduced concentration of the amino acid phenylalanine. In both diet A0 and A1, ornithine and β-alanine were omitted, as described by Karley et al. (2002). Diet B (Febvay et al. 1999) was developed for pea aphids based on the total amino acid profile of alfalfa, *Medicago sativa* L. Diets C and D were the young and old green peach aphid diets, respectively, described by Karley et al. (2002). Concentrations of sucrose, vitamins, and minerals (Table 2) were the same for each diet (Febvay et al. 1988).

Table 1. Amino acid composition (in millimolar) of the aphid artificial diets used to rear soybean aphids

L-Amino acid	Diet A0[a]	Diet A1[a]	Diet B[b]	Diet C[c]	Diet D[c]
Ala	20.1	20.1	5.4	4.9	4.2
Arg	14.1	14.2	3.5	5.7	6.6
Asn, H_2O	19.9	19.9	179.0	7.4	4.9
Asp	6.6	6.6	11.9	15.6	14.7
Cys	2.4	2.4	1.6	2.2	1.9
Glu	10.2	10.2	4.9	20.3	11.5
Gln	30.5	30.5	5.9	36.6	24.9
Gly	22.2	22.2	1.7	2.1	1.5
His, HCl, H_2O	6.5	6.5	1.7	1.4	2.7
Ile	12.6	12.6	4.0	3.4	8.0
Leu	17.7	17.7	3.8	3.4	9.3
Lys, mono HCl	19.2	19.2	2.2	5.2	7.8
Met	4.9	4.9	0.8	2.2	2.9
Phe	10.3	3.8	3.0	4.6	8.7
Pro	11.2	11.2	4.3	4.9	4.2
Ser	11.8	11.8	10.4	8.1	5.7
Thr	10.7	10.7	6.8	9.6	9.1
Trp	2.1	2.1	1.4	5.3	8.9
Tyr	2.1	2.1	1.1	0.6	1.4
Val	16.3	16.3	5.9	6.2	2.9

[a] Febvay et al. (1972).
[b] Febvay et al. (1999).
[c] Karley et al. (2002).

Diets were prepared in 1,000-ml volumes by adding the correct amount of amino acids, vitamins, and minerals to a flask filled to two thirds of the total desired volume with an 845 mM solution of sucrose. The pH of the solution was then adjusted to 7.5 with KOH, and it was brought to a final volume of 1,000 ml. The diet solution was then filter-sterilized by passing it through a 0.45-μm Millipore filter, divided into 20-ml aliquots, and stored at −20°C for no longer than 3 mo. Distilled-deionized water was used in all solutions.

For each of the five aphid artificial diets, eight replications were performed. For each replication, 10

Table 2. Mean developmental time from nymph to adult, fecundity, and longevity of soybean aphids reared on different food sources

Food source	Mean developmental time (d)[a]	Mean fecundity (nymphs produced/ aphid)[a]	Mean longevity (d)[a]
Detached leaves	4.5 ± 0.7a	15.1 ± 2.6b	15.0 ± 2.1c
Water under parafilm			2.9 ± 0.4a
Leaves under parafilm	6.7 ± 0.7b	12.2 ± 1.5a	13.1 ± 1.9b
Leaf extract under parafilm	7.0 ± 0.9b	11.6 ± 1.3a	12.8 ± 2.5b

Aphid nymphs reared on a diet sachet containing only water did not develop into adults or produce offspring.

[a] Values within a column are not significantly different if they share the same letter, as determined by the least significant difference test ($P = 0.05$).

Table 3. Mean developmental time from nymph to adult, fecundity, longevity, and percentage of maturation of the first generation of soybean aphids reared on five different artificial diets

Diet	Mean developmental time (d)[a]	Mean fecundity (nymphs produced/aphid)[a]	Mean longevity (d)[a]	Maturation (%)[a]	Total generations produced[a]
A0	6.7 ± 1.0c	3.7 ± 0.9d	8.5 ± 0.8e	88.8c	3.4 ± 1.1d
A1	7.0 ± 0.7d	5.0 ± 0.7c	9.9 ± 0.8c	91.3b	4.5 ± 0.8c
B	6.1 ± 0.8b	8.6 ± 0.9b	10.4 ± 0.9b	93.8b	7.8 ± 0.7b
C	5.7 ± 0.7a	11.2 ± 0.8a	12.0 ± 0.8a	96.3a	12.3 ± 0.9a
D	6.9 ± 0.8cd	5.0 ± 0.9c	9.6 ± 0.7d	86.3d	4.6 ± 0.9c

The total number of generations supported by the diet is also reported as the mean of the eight replications per diet, rounded to the nearest whole number.

[a] Values within a column are not significantly different if they share the same letter, as determined by the least significant difference test ($P = 0.05$).

aphid nymphs (< 6 h old) were transferred onto a diet sachet by using a sable-hair brush. Diet sachets were changed two times per week throughout the course of the study. Aphid mean developmental time from larvae to adult, fecundity, and mortality were measured in days throughout the experiment. Percentage of maturation was determined as the percentage of aphid nymphs that survived to become adults.

The first 10 aphid nymphs deposited per generation were removed and transferred to a new diet chamber to assess the ability of subsequent generations to develop on the artificial diet being tested. This was continued until the colony collapsed (all aphids had died), and the mean number of generations supported by each diet was determined.

Statistical Analysis. Statistical analysis was performed with SAS 9.1 (SAS Institute 2007). The least significant difference test at $P = 0.05$ was used to separate treatment means when they were determined to be significantly different ($P < 0.05$) in the one-way analysis of variance.

Results

Initial Feeding Studies. Aphids feeding on detached Williams 82 soybean leaves had the shortest mean developmental time, highest fecundity, and lived the longest of all groups tested (Table 2). The mean developmental time, fecundity, and mortality were not significantly different for aphids feeding on diet sachets containing detached leaves or soybean leaf extract. Soybean aphids feeding on a diet sachet containing only water did not reach maturity or produce any offspring, and their mean longevity was significantly lower than aphids in the other three treatment groups (Table 2).

Artificial Diet Studies. Aphid mean development time, the amount of time required for a nymph to become an adult, ranged from 5.7 to 7.0 d (Table 3). Aphids on diet C had the shortest mean developmental time (5.7 d), followed by aphids on diet B (6.1 d). The percentage of aphids that reached adulthood ranged from 86.3% in diet D to 96.3% in diet C. Aphids on diets A1 (7.0 d) and D (6.9 d) had the longest mean developmental times, and they were not significantly different from one another. Similarly, aphids on diets D and A0 (6.7 d) had significantly longer mean developmental times than the two best diets.

Fecundity on the five diets tested ranged from 3.7 to 11.2 nymphs produced by each adult aphid over the course of its life span (Table 3). Fecundity was highest on diet C (11.2 nymphs per aphid), and it was 67% higher than the diet with the lowest fecundity, diet A0 (3.7 nymphs per aphid). Diet B had the second-highest fecundity (8.6 nymphs per aphid), followed by diets A1 (5.0 nymphs per aphid) and D (5.0 nymphs per aphid), which were not significantly different from one another.

Aphids reared on diet C had the highest longevity of all diets studied, 12.0 d (Table 3). This was followed by aphids on diet B (10.4 d), diet A1 (9.9 d), and diet D (9.6 d). Aphids reared on diet A0 had the shortest life span, 8.5 d, a decrease in longevity of 29% from aphids reared on diet C. The longevity values for all diets were significantly different from one another.

The total number of soybean aphid generations sustained on the five artificial diets tested ranged from 3.4 generations on diet A0 to 12.3 generations on diet C (Table 3). Diet B (7.8 generations) supported significantly fewer generations than diet C, but still allowed more generations of aphids to develop than did the other three diets. Diets A1 (4.5 generations) and D (4.6 generations) supported the second-lowest number of aphid generations, and they were not significantly different from one another.

Discussion

Initial feeding studies determined that soybean aphids were able to probe and feed through a Parafilm M membrane, an essential part of the diet chamber used to test artificial diets. On average, soybean aphids that were allowed to feed on soybean leaves or soybean leaf extract encased in Parafilm M lived 9.9 d longer than soybean aphids that were fed only water. This evidence suggests that the aphids were able to probe through the membrane to obtain food, because they lived significantly longer than aphids that starved. Soybean aphids feeding on detached leaves that were not covered by Parafilm M had the highest longevity and fecundity, and the lowest mean developmental time of all groups tested, suggesting that the Parafilm M membrane did decrease the ability of soybean aphids to feed.

In addition to the initial feeding studies, the ability of soybean aphids to feed on five previously described aphid

Fig. 1. Soybean aphid diet chambers. The dish in the top left corner contains a detached Williams 82 soybean leaf; the dish in the top right corner contains a diet sachet filled with 2 mL water; the dish in the bottom left corner contains a diet sachet filled with a detached Williams 82 soybean leaf; and the dish in the bottom right corner contains a diet sachet filled with 2 mL Williams 82 soybean leaf extract.

artificial diets was tested. Diets had the same concentration of sucrose, vitamins and minerals, and they varied only in the concentration of amino acids. Three of the diets studied (A0, A1, and B) were initially developed for the pea aphid, an aphid species that also feeds solely on legumes. The remaining two diets studied (C and D) were originally developed for the green peach aphid, a polyphagous species. Diets C and D also had been used to rear *Macrosiphon euphorbiae* (Thomas).

Diets C and D were developed for the green peach aphid, and they were based upon the amino acid analysis of the phloem of young and old potato (*Solanum tuberosum* L.) plants, respectively. Green peach aphids are known to prefer young potato plants, and to perform poorly on older potato plants that have already produced tubers (Karley et al. 2002). Soybean aphids reared on diet C performed better than soybean aphids reared on any of the other four diets tested, producing 12.3 generations of aphids. In contrast, soybean aphids reared on diet D performed similarly to soybean aphids reared on diet A1, with a total of 4.5 generations.

The concentrations of asparagine, glutamic acid, glutamine, glycine, and valine in diet D were reduced in comparison to diet C. The concentration of valine in diet D was the lowest out of all the diets tested, and they could have been a limiting factor in soybean aphid performance. The differences in concentrations of glutamic acid and glutamine between diet C and diet D may have contributed to better soybean aphid performance.

Artificial diet B was intermediate in supporting soybean aphid development. Like diets A0 and A1, diet B also was based on the amino acid composition of alfalfa phloem. The concentrations of all amino acids in diet B, with the exception of aspartic acid and asparagine, were lower in comparison to diets A0 and A1. Soybean aphids reared on diet B reproduced for 7.8 generations, and their fecundity and longevity were significantly higher than that of soybean aphids reared on diets A0 and A1. Similarly, the mean developmental time from nymph to adult was significantly decreased in comparison with diets A0 and A1. The results obtained from this diet experiment suggest that soybean aphids need more aspartic acid and asparagine than is provided by diets A0 and A1, because these two amino acids were present at higher concentrations in diet B. Aphids are able to increase their ingestion of a food source that is deficient in one or more amino acids, but by doing so they can compensate only for a slight deficiency (Febvay et al. 1999). These factors could explain how a reduction in all amino acids other than aspartic acid and asparagine resulted in an improvement in the performance of soybean aphids on diet B in comparison with diets A0 and A1.

Diets A0 and A1 were based on the amino acid analysis of pea aphids grown on broad beans, *Vicia*

faba L. Soybean aphids reared on these diets performed very poorly, with diets A0 and A1 supporting 3.4 and 4.5 generations of aphids, respectively. This suggests that the amino acid balance of the diet is not sufficient to maintain soybean aphid development. Aphid nymphs produced by females that were raised on the proper host plant are often able to survive and reproduce on an artificial diet that is lacking in amino acids, but subsequent generations are not able to develop properly (Dadd and Krieger 1968). In this case, the very low number of soybean aphid generations produced on diets A0 and A1 suggests that the balance of amino acids in the diets allows one generation of aphids to develop, but nutritional deficiencies cause a decrease in the longevity and fecundity of the aphids' offspring.

The results obtained in this study suggest that soybean aphids are able to survive and reproduce on the diet proposed by Karley et al. (2002). Twelve successive generations of soybean aphids were reared on this diet, although to maintain a soybean aphid colony on an artificial diet for long periods of time, improvement is still needed.

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