ECOLOGY AND BEHAVIOR

Effect of Nitrogen Fertilization on *Aphis gossypii* (Homoptera: Aphididae): Variation in Size, Color, and Reproduction

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ABSTRACT The effect of nitrogen fertilization on *Aphis gossypii* Glover color and size, fertility, and intrinsic rate of increase \( (r_m) \) was studied on cotton plants. Nitrogen fertilization treatments consisted of 0, 50, 100, and 150% of the agronomic recommended level. Adult and nymph densities, as well as \( r_m \), were positively correlated with nitrogen fertilization. Aphid body length, head width, and darkness of color were recorded in populations on cotton plants fertilized with 100% nitrogen or with no nitrogen fertilization. Aphids on nitrogen-fertilized plants were significantly bigger and darker. All body size and darkness of color measurements were positively correlated with aphid fecundity. It was also found that the nutritional quality of the host plant on which the parent generation feeds has a stronger effect on the aphids than that of the quality of their own food plants. This phenomenon may dampen the effect short-term fluctuations in host plant quality have on aphid performance. Results are discussed in the context of aphid population biology, aphid—plant interactions and aphid population management.

KEY WORDS *Aphis gossypii*, nitrogen fertilization, morphology, fecundity, cotton, aphid—plant interactions

ONE OF THE most striking characteristics of many aphid species is the remarkable morphological changes they undergo. Aphids of the same species can be highly polymorphic, enough to have misled some taxonomists to regard them as different species (Leclant and Deguine 1994). The cotton aphid, *Aphis gossypii* Glover, a major pest of cotton and many other crop plants around the world (Leclant and Deguine 1994), has been reported to undergo seasonal color and morphological changes (Setokuchi 1981, Wilhoit et al. 1993, Rosenheim et al. 1994, Watt and Hales 1996). In spring populations, cotton aphids are often darker and may be twice as large as individuals in summer. These aphids exhibit shorter development time and higher fecundity compared with aphids during the summer (Rosenheim et al. 1994). The summer aphids are sometimes referred to as “yellow dwarves” (Watt and Hales 1996). Such variation in fecundity results in shorter generation time and higher intrinsic rate of population increase \( (r_m) \) in spring. These morphs, however, are not distinct, and intermediate morphs are often found between the two extremes (Wilhoit and Rosenheim 1993, Rosenheim et al. 1994, Wool and Hales 1997).

Cotton aphid populations in cotton fields in Israel, as well as in North America and other parts of the world, go through a rapid increase at the beginning of the season (spring populations), and collapse during the summer (Broza 1986, Rosenheim 1995). In some fields, however, spring populations do not collapse but proliferate throughout the summer, causing severe economic damage, particularly after bolls have begun to open and the lint is exposed. The reason for this phenomenon is unclear (Broza 1986).

One of the most important factors influencing the performance of herbivorous insects is nitrogen level in their diet (Douglas 1993). Often, nitrogenous compounds are scarce in plant tissue, particularly in phloem sap (Mattson 1980). Therefore, sucking insects such as aphids show a strong response to nitrogen level in their host plants (van Emden 1996). In agroecosystems, nitrogen fertilizers are an important source of nitrogen (van Emden 1996). High dosages of nitrogen fertilizers have been reported to have various effects on cotton aphid populations on cotton. Comparison between cotton aphids on fertilized and non-fertilized cotton plants revealed that nitrogen fertilization had a significant positive effect on aphid fecundity, but had no effect on aphid weight (Rosenheim et al. 1994). In another study in cotton, nitrogen fertilization in the field had no effect on the number of cotton aphids per leaf (Slosser et al. 1997). Relatively little is known about the effect of leaf nitrogen level on aphid morphology. Because many aphid species (cotton aphid included) exhibit viviparous reproduction, the effect of nitrogen content in the parent’s diet on offspring morphology should also be considered. In the current study, we tested the effect of nitrogen fertilization on morphology (uni- and bi-generational), fecundity, and population growth of *A. gossypii* in cotton.
Materials and Methods

Cotton plants, Gossypium hirsutum L. (‘Pima F-177’, Hazera, Brurim, Israel), were sown in 13- to 15-cm-diameter pots in a substrate composed of peat (60%) and volcanic gravel (40%). Plants were irrigated two or three times daily. One week after emergence, the seedlings in each pot were thinned, so that only one plant remained in each pot. Two grams of KCl (as a source of potassium) and 2 g of slow-release phosphorus fertilizer (Kedem Chemicals, Ashdod, Israel) were applied per pot. Nitrogen fertilizer was added 1 wk later. A slow-release 40% nitrogen fertilizer (Multicote 40-0–0, Haifa Chemicals, Haifa, Israel) was used. The optimal nitrogen level found for Pima cotton in Israel was found to be 5.5 g per plant (Shemesh et al. 1997). Because of the use of a substrate with low water content, particularly for a relatively short period, the nitrogen level was elevated, and the optimum was set to 5 g N per plant. Plants used for aphid culture were fertilized with a rich, slow release fertilizer (16-8-12 + microelements, Osmocote Plus, Scotts Europe, Heerlen, The Netherlands). In some experiments, leaf area was measured within 4 h of leaf excision, using a leaf area meter (AT Area Meter, Delta-T Devices, Cambridge, England). Leaf nitrogen level was determined by cutting out leaf discs. Discs were then dried at 60°C for at least 24 h, and digested overnight in sulfuric acid. After heating at 200°C for 30 min to assure complete digestion, hydrogen peroxide was added to reduce all nitrogen compounds in the sample to ammonium (NH₄). Total reduced nitrogen was determined by addition of phenol and hypochloride reagents to create a phenol-ammonium complex. Total nitrogen concentration was measured using a spectrophotometer (Ultrospec 4050, Biochrom, Cambridge, England) at 640 nm (Smith 1980).

Cotton aphids were collected from commercial cotton fields in the Judea foothills (latitude: 31° 50’ N, longitude: 34° 50’ E) in Israel. Aphids were grown on cotton plants in a climate-controlled room at 25 ± 2°C and a photoperiod of 16:8 (L:D) h. The plants used for aphid cultures were replaced weekly.

Experiments were conducted at 25 ± 2°C and a photoperiod of 16:8 (L:D) h. Each plant was placed in a transparent plastic cage (60 by 40 by 30 cm.), in which one side was replaced with a fine mesh for ventilation. Irrigation could be applied without opening the cages that remained sealed throughout the experiment.

To test the effect of nitrogen fertilization on aphid density, plants were treated with four nitrogen levels: 0, 50, 100, and 150% of the optimal level. The youngest expanded leaf of each plant was infested with 10 adult females (1-2 d old). Eight days later, plants were removed from the cages, and the adults and nymphs on each leaf were counted. Aphid density was determined by dividing aphid count by leaf area. Intrinsic rate of population increase (rm) was also calculated (Dixon 1987).

To measure aphid fecundity, the same four treatments were used. Five adult females (1-2 d old) were placed on each plant in a 15-cm² clip cage (d = 4 cm; modified after Addicott 1981). Twenty-four hours after infestation, cages and adults were removed, and nymphs were counted.

The size and color were determined for aphids on plants treated with 0 or 100% nitrogen. Five adult aphids were placed on each plant. Eight days later, aphids were collected and video taped. The video captures were analyzed using NIH Image software (National Institutes of Health, Bethesda, MD, rsbweb.nih.gov). Aphid body length (front of head to cauda) and head width (between lateral borders of eyes) were measured. Measurement of darkness of aphid color was performed on the abdomen, and was quantified on a scale of 0 (white) to 255 (black).

To test for correlation between cotton aphid morphology and fecundity, the measured morphological characters were averaged on each plant and correlated with the number of nymphs on that plant.

To determine the cumulative effect of nitrogen fertilization on the aphids over two consecutive generations, adult females that developed on 0 or 100% nitrogen treated plants were used to infest new plants of the two nitrogen treatments (five females per plant). A total of 12 plants in four treatments was used (three replications in each treatment). The same morphological measurements were taken 8 d later.

For data analysis, t-tests and analysis of variance (ANOVA) were used (SAS Institute 1995). Data were tested for homogeneity of variances (Levene’s test, Milliken and Johnson 1984). When variances were found to be not homogeneous, Welch ANOVA, Wilcoxon or Kruskal–Wallis tests were used (SAS Institute 1995). A protected Tukey–Kramer test was used for mean separation (SAS Institute 1995). Significance level was set at P = 0.05.

Results

Nitrogen treatment was found to have a significant effect on leaf nitrogen level (F = 21.13; df = 3, 197; P < 0.001). This effect reached a plateau, as expected, at the 100% level (Fig. 1). Nitrogen fertilization had no significant effect on potassium (F = 2.32; df = 3, 197; P = 0.076) and phosphorous (F = 2.01; df = 3, 197; P = 0.114) levels in the leaves.

Fig. 1. Influence of nitrogen fertilization on leaf nitrogen content (means ± SD). Bars with the same letter do not differ significantly (P < 0.05; Tukey–Kramer test).
Aphid populations attained significantly higher densities on plants fertilized with high compared with low nitrogen levels ($F = 5.41; \text{df} = 3, 23; P < 0.007$) (Fig. 2). Although nymph and adult densities were positively correlated with nitrogen level, the effect was stronger for the nymphs (nymphs: $R^2 = 0.41, P = 0.001$; adults: $R^2 = 0.21, P = 0.023$). These data were used to calculate the average intrinsic rate of increase. A significantly higher rate of population increase was found on plants treated with high than low nitrogen fertilization rates ($r_m = 0.21 \pm 0.04a, 0.32 \pm 0.04ab, 0.35 \pm 0.03bc, and 0.46 \pm 0.02c$, respectively, for aphids on plants receiving 0, 50, 100, and 150% nitrogen fertilization). These differences are reflected in the predicted effect of nitrogen fertilization on population size (Fig. 3).

Nitrogen fertilization level had a significant effect on female fecundity ($F = 4.43; \text{df} = 3, 8; P = 0.041$), because high numbers of offspring were produced per female on plants treated with 150% nitrogen (Fig. 4). The general trend of increase in fecundity with the increase in nitrogen fertilization was confirmed by linear regression ($R^2 = 0.59, P = 0.003$).

Aphids that developed on 100% nitrogen fertilized plants were longer ($t = 9.94, n = 99, P < 0.001$), had wider heads ($t = 6.50, n = 99, P < 0.001$), and were darker in color ($t = 6.31, n = 99, P < 0.001$) compared with aphids reared on nitrogen-deprived plants (Fig. 5). When these parameters were measured for two consecutive aphid generations (Table 1), results show that, as expected, aphids that were reared for both generations on well fertilized plants were longer ($F = 43.54; \text{df} = 3, 190; P < 0.001$), wider (Welch ANOVA; $F = 23.20; \text{df} = 3, 186; P < 0.001$), and darker (Welch ANOVA; $F = 39.26; \text{df} = 3, 190; P < 0.001$) compared with those reared on unfertilized plants (Fig. 6). However, aphids that developed on fertilized plants, but their mothers developed on unfertilized plants, were significantly shorter, narrower ($t = 5.17, \text{df} = 86, P < 0.001$), and of a lighter color ($t = 8.59, \text{df} = 86, P < 0.001$) compared with aphids reared on nitrogen-deprived plants (Fig. 5).
0.001) than aphids that developed on unfertilized plants but whose mothers had developed on fertilized ones (Fig. 6). These morphological differences were positively correlated with female fecundity (aphid body length: $R^2 = 0.81$, $P < 0.001$; head width: $R^2 = 0.50$, $P = 0.010$ and darkness of color $R^2 = 0.61$, $P = 0.003$).

### Table 1. Effects of nitrogen fertilization during two aphid generations on aphid size and darkness of color

<table>
<thead>
<tr>
<th>Nitrogen level, %</th>
<th>First generation</th>
<th>Second generation</th>
<th>No. of aphids</th>
<th>Body length (mm ± SD)</th>
<th>Head width (mm ± SD)</th>
<th>Darkness (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>1.139 ± 0.022a</td>
<td>0.270 ± 0.003</td>
<td>166.9 ± 2.4</td>
</tr>
<tr>
<td>0</td>
<td>100%</td>
<td>46</td>
<td>224 ± 0.019b</td>
<td>0.281 ± 0.003</td>
<td>168.0 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>0</td>
<td>41</td>
<td>1.319 ± 0.029b</td>
<td>0.300 ± 0.003</td>
<td>188.7 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>100%</td>
<td>74</td>
<td>1.444 ± 0.015d</td>
<td>0.301 ± 0.002</td>
<td>189.6 ± 1.6</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA, Welch ANOVA, Welch ANOVA

$F = 4.54$, $F = 23.20$, $F = 39.26$, $\text{df} = 3.190$, $\text{df} = 3.186$, $\text{df} = 3.190$, $P < 0.001$, $P < 0.001$, $P < 0.001$

Aphid darkness of color ranges from 0 = white to 255 = black.

### Discussion

Host plant quality is known to be an important factor affecting aphid morph production (e.g., frequency of alates), development rate, and fecundity (Dixon 1987). This study illustrates that such effects of nitrogen fertilization are correlated with aphid color and size, and that these effects could be detected across generations.

Like our study, others also reported cotton aphid color to be correlated with aphid weight (Rosenheim et al. 1994) and body size (Ebert et al. 1998). In turn, aphid body size is often correlated with fecundity and intrinsic rate of population increase (Dixon 1991). However, the relationship between body color and cotton demography could be influenced by environmental conditions, such as temperature (Setokuchi 1981).

It is unlikely that the recorded changes in aphid morphology are due to aphid crowding rather than to nitrogen fertilization. Our data indicate that at the time measurements were taken (8 d after infestation) the rate of population growth was still increasing and not decreasing, as expected in crowded populations. Furthermore, the appearance of winged aphids (alates) would indicate aphid crowding (Kawada 1987). Yet, no alates were formed during the experiments.

An increase in aphid performance can also be the result of reduced plant defense. The primary defense compound in cotton plants is gossypol ($C_{29}H_{30}O_{8}$), which contains no nitrogen. According to the carbon-nutrient balance hypothesis reviewed by Price (1997), gossypol levels in cotton leaves decrease in response to nitrogen fertilization. Such reduction in plant defense in response to nitrogen fertilization may underlie the recorded differences in aphid traits between the 100 and 150% treatments because leaf nitrogen levels did not differ in plants receiving these fertilization levels. Other factors, such as leaf water content, may be responsible for the increase in aphid fecundity without detecting a similar increase in leaf nitrogen level (e.g., between 100 and 150% treatments). Yet, phosphorus and potassium levels in the leaves did not differ between fertilization treatments (Nevo 1999).

Variations in the intrinsic rate of population increase could be attributed to three main factors: (1)
development time (from first instar to adulthood); (2) fecundity, i.e., the number of offspring per female per time unit; and (3) longevity, reflecting the length of the reproductive period (Dixon 1987). The current experiments provide evidence on the effects of nitrogen fertilization on the increase in aphid fecundity. An indication of the effect of nitrogen on female longevity and nymph development rate is provided by the significantly higher adult count on well fertilized compared with unfertilized plants. This difference could be due to lower mortality of adults or faster nymph development rate on fertilized plants.

Our data show that the parent’s nutrition had a greater effect on the cotton aphid than its own nutrition. If so, these results are of great importance for the understanding of aphid population biology. Wide fluctuations in foliage nutritional value are common in nature and occur over both time and space. For example, leaf nitrogen content changes during the growing season and older leaves often have lower nitrogen levels than young ones (Matton 1980). Yet, the effects of these fluctuations on aphid fitness may be obscured because of the unique reproductive biology of many species in this group. In Israel and elsewhere, cotton aphids are parthenogenetic and viviparous (Avidov and Harpaz 1969, Freidberg et al. 1989)—the embryos of the next generation already develop in the ovaries of a newborn aphid. Therefore, aphid morph formation is, in part, the result of accumulating, long-term changes in plant quality. This phenomenon may dampen the effect of short-term fluctuations in host plant quality have on aphid performance.

As mentioned earlier, the reasons for the eruptions of cotton aphid populations in cotton fields are unknown. These population eruptions are often correlated with the presence of large and dark morphs of the aphid (Rosenheim et al. 1994). In view of the data presented here and earlier studies (reviewed by Slosser et al. 1997), it is possible that plant nitrogen content is at least partially responsible for the induction of such population eruptions.

Nitrogen fertilizers are used in many crops throughout the world. Excess use of such fertilizers contributes to the pollution of water bodies and leads to further environmental damage (Smil 1997). Our results show that there is a substantial difference in \( r_m \) between aphid populations on plants fertilized with 150% nitrogen versus plants fertilized with 100% nitrogen or less. Thus, careful application of nitrogen fertilizers so that the optimal levels (100%) are not exceeded could significantly reduce the number of aphids and thus their damage to cotton. In addition, reduction of the amount of nitrogen applied would minimize levels of nitrogen pollution as well as cut cotton production costs.

Because nitrogen fertilization affects aphid morphological traits and these in turn are positively correlated with fecundity, color and size, such characteristics should be considered in their population management. The obtained data could be used to forecast the behavior of aphid populations and be incorporated into decision-making tools for their control.

That nitrogen fertilization enhances aphid performance should not be surprising. However, its cumulative effect (over several generations) on aphid color and size provides new insights into their population biology and to aphid–plant interaction in general.

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