# The influence of black bean aphid, *Aphis fabae* Scop., and its honeydew on the photosynthesis of sugar beet

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## Summary

The effect of black bean aphids on the photosynthesis of sugar beet plants was studied under glasshouse and field conditions. The presence of up to several hundred aphids per leaf had no significant effect on $CO_2$ exchange rates over a range of light intensities between complete darkness and light saturation. Artificially prepared honeydew, sprayed onto leaves in the same amounts and composition as was found on severely aphid-infested plants, covered 30% of the stomata on the upper epidermis but did not significantly alter the rate of photosynthesis of these leaves in the light or the rate of respiration in the dark. The stomata on the lower epidermis were uncovered and functional. High pressure liquid chromatography of aphid-produced honeydew detected 20 different amino-acids. Three amino-acids, aspartic acid, glutamic acid and glutamine, made up the bulk of the amino-acid weight in the honeydew produced on young plants, up till the 8 leaf-stage. In the 10 to 12 leaf-stage, several different amino-acids occurred in substantial amounts. The amino-acids to sugars ratio of the honeydew produced by the aphids decreased strongly as the sugar beet plants aged: from 1:6 in plants with 3 or 4 leaves to 1:25 in plants having 10 to 12 leaves.

## Introduction

The black bean aphid, *Aphis fabae* Scop., is the most important pest of sugar beet in Eastern Europe (Weismann & Vallo, 1963; Hurej, 1984). Sugar beet seedlings are infested in the spring by winged viviparous females migrating from the winter hosts, predominantly spindle trees, *Euonymus europaeus* L. The aphid populations retard the growth of the young plants and heavy attacks may kill them. On older plants, colonies of several thousand aphids may occur, but in this growth stage there is little damage. The damage caused to young plants is ascribed to a variety of injury mechanisms (Jones & Dunning, 1972; Cammell, 1981): [1] consumption of phloem contents (sugars and amino-acids), [2] leaf curling which diminishes light interception, [3] morphogenetic, metabolic and translocational disorders as well as energy demanding defense reactions induced in the host, and [4] fouling of the leaf surface with honeydew, which may affect leaf functioning in several ways. This paper aims at quantifying the fourth mechanism.

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Both detrimental and stimulatory effects of aphids on the photosynthesis of host plants have been described. Detrimental effects occur in winter wheat. Rabbinge *et al.* (1981) measured reduced maximum rates of photosynthesis in wheat flag leaves one day and one week after honeydew application in the glasshouse. The decreased rate of photosynthesis after one day was ascribed to hampered gas exchange as a result of clogging of stomata. Accelerated leaf senescence was held responsible for the long-term effect of honeydew on leaf photosynthesis (Vereijken, 1979; Rabbinge *et al.*, 1981). In the field short-term effects were not found, however. Field experiments by Rossing & van de Wiel (1990) confirmed the reduction of maximum photosynthesis rates after 14 days but did not demonstrate rapid effects. Honeydew stimulates the growth of saprophytic phyllosphere fungi (Ajayi & Dewar, 1983; Fokkema, Riphagen, Poot & de Jong, 1983), which, if present in great densities, hamper photosynthesis by absorbing light. Honeydew can also increase the virulence of perthotrophic fungi (Dik, 1990). Three different aphid species cause major reductions in leaf photosynthesis in pecan tree, *Carya illinoensis* (Wangenh) K. Koch (Wood, Tedders & Thompson, 1985).

Increased photosynthesis was observed in broad bean (*Vicia faba* L.) and cowpea (*Vigna unguiculata* (L.)) by Hawkins, Aston & Whitecross (1987), after 6–9 days of feeding by *Aphis craccivora* Koch or *Acyrthosiphon pisum* (Harris). These authors suggested that the stimulation may be explained by increased sink demand for photosynthesis products, while the apparent excess production was consumed by the aphids. Way & Cammell (1970) forwarded the same hypothesis to explain the stimulation of photosynthesis in cabbage leaves infested with cabbage aphid, *Brevicoryne brassicae*. Dixon (1971*b*) reported increased leaf productivity in sycamore trees (*Acer pseudoplatanus* (L.)) infested with sycamore aphid *Drepanosiphum platanoides* (Schr.). In lime tree (*Tilia x vulgaris* Hayne) infested with lime aphid (*Eucallipterus tiliae* L.), such stimulus occurred also, not in the year of aphid infestation but in the year after it (Dixon, 1971*a*).

Overall, negative effects of aphids on plant photosynthesis have been reported more often than stimulation. The size of the effects of aphid infestation on leaf photosynthesis and the mechanisms involved seem to depend strongly on the details of the system under study, such as host plant species, aphid species, growth stage of the host plant, light, water and nutrient status and the duration and severity of the aphid infestation.

In sugar beet, the effects of feeding by black bean aphids and of honeydew deposits by this aphid on leaf photosynthesis have not been studied. The purpose of our study was to ascertain the nature and magnitude of these effects in order to obtain a mechanistic understanding of how black bean aphid causes damage to sugar beet. Such insight is useful for defining flexible damage thresholds that take account of the conditions under which the aphid infestation occurs.

## Materials and Methods

Measurements were made in the glasshouse and in the field in the spring and summer of 1988. In the glasshouse, individual sugar beet plants, cv. Salohil, were grown on a half strength Hoagland nutrient solution in 70 litre aerated containers. The glasshouse was maintained at a temperature of 18–20°C and a relative humidity of 65%. No additional illumination was given. The field measurements were made in a sugar beet crop cv. Salohil, grown on a heavy river clay soil at an experimental farm ('de Bouwing') in Randwijk near Wageningen, the Netherlands. On 15 May, a homogeneous group of 150 'representative' plants in the 2 true leaf-stage were marked with bamboo sticks. In the glasshouse and in the field three experimental treatments were given:

[1] *Infestation with A. fabae.* In the glasshouse, plants at the 3–4 leaf-stage were infested with two winged *A. fabae* from spindle bush on 28 May. Infestation in the field relied on natural immigrants as numerous winter hosts of *A. fabae* were present in the neighbourhood. Those plants that had been colonised at or before the 6 leaf-stage (25 May) were included in the treatment group. The incipient aphid colonies were sheltered from predators by sticky barriers. These barriers were removed after the aphid colonies were established in mid-June.

[2] *Application of artificial honeydew.* To study the effect of honeydew separately from aphid feeding, an artificial honeydew solution was prepared and sprayed with a hand sprayer onto leaves of aphid-free plants. (For composition of this solution, see below.) Artificial honeydew was applied in such quantities that a leaf coverage was obtained that looked similar to that observed on the leaves of aphid-infested plants. The applications were made simultaneously in the glasshouse and in the field on 2, 9, 13, 21 and 28 June and on 9 July. The precise concentration applied on the leaf surfaces in the field was assessed afterwards (see below).

[3] *Control.* In the glasshouse, any settling aphids were removed with a brush, while in the field, accidentally colonising aphids were killed with a spray of Pirimor (0.5 g of pirimicarb per litre). Each treatment was applied to at least 10 plants.

### Composition of natural and artificial honeydew

*Aphis fabae* were reared outdoors on 10 sugar beet plants, which had been sown at the end of April in 25 cm diameter pots with standard pot soil. Each plant was infested with four aphids from spindle bush on 12 May when the plants were at the 2 leaf-stage. Honeydew was collected on sheets of aluminium foil placed on the pot surface from 15 to 16 May (3–4 leaf-stage), on 26 May (6–8 leaf-stage) and from 31 May to 4 June (10–12 leaf-stage). The crystallised honeydew was dissolved from the foil into a small amount of demineralised water, using a brush. The honeydew solution was filtered through paper to remove exuvia and dirt and then stored at −18°C. Amino-acids were analysed by high pressure liquid chromatography (HPLC), using the pico tag method (Millipore-Waters). Total sugars, not differentiating between monosaccharides, disaccharides or higher sugars, were determined colorimetrically according to a modified Nelson & Somogy method (Vertregt & Verhagen, 1979). Following each determination a 25% artificial honeydew solution with the same dry matter composition as the real honeydew was prepared, using sucrose as the only sugar. Each solution was used in two applications (see above).

### Assessment of honeydew concentration on the leaves

Five representative leaves were collected in the field from each treatment both 1 day after and 1 day before each application. Honeydew was washed from the leaf using a small amount of demineralised water and a brush. The honeydew solution was filtrated, stored and analysed as described above. The area of the collected leaves was determined with a Licor 3100 electronic leaf area meter.

### Assessment of stomatal blocking by honeydew

Five leaves heavily covered with natural or artificial honeydew were collected in the glasshouse. From each leaf a silicone rubber imprint of the upper and lower epidermis was made (G. A. Pieters and M. E. van den Noort, personal communication). The number of stomata on 15 randomly-chosen areas of 6.05 mm$^2$ per imprint were counted using a Leitz ortholux light microscope at a magnification of 240×. The counts were made using reflected

light provided by Leitz ultropak object lenses. The percentage of stomata covered with honeydew was determined on four areas per imprint.

## Photosynthesis measurements

Leaf photosynthesis was measured on 3, 10, 18 and 24 June in the glasshouse and on 10 July in the field, using an LCA-2 portable open system for photosynthesis measurement (The Analytical Development Co. Ltd, Hoddeson, UK, 1985). Measurements in the field were made on a sunny day on 10 July, under natural light. Measurements in the laboratory were made under artificial illumination by six Philips 400 W HPI lamps per m². Infra-red radiation was removed by a water bath between the lamps and the plants. Saturating light intensities of about 300 W m$^{-2}$ (photosynthetically active radiation with 400–700 nm wavelength; PAR) were obtained in the field and in the laboratory. On each date, three leaves of one plant for each treatment group were examined: a young still expanding leaf, a recently expanded leaf and a fully mature leaf. On aphid-infested plants, severely-infested leaves were selected (up to 600–1500 aphids per leaf; the aphids were not removed), while on honeydew sprayed plants, strongly-covered leaves were chosen. Ambient air with 340 to 360 ppm $CO_2$ and 30% to 50% r.h. was led through the leaf chamber. Leaf temperature in the chamber varied from 25°C to 30°C. For each leaf five or six measurements of $CO_2$ exchange were made, the first one at full illumination and the next ones at increasing degrees of shading. After at least one half hour adjustment to the highest light level, measurements were recorded after adaptation of the leaf to the lowered level, typically after 2 min. On 9 June, additional measurements were made with an open system in the laboratory, modified after Louwerse & van Oorschot (1969). Measurements were taken in the usual way for this system, leaving 30 to 45 min adaptation time after changing the light intensity.

Photosynthetic parameters of individual leaves were calculated by fitting negative exponential saturation curves (Goudriaan, 1982) to the measurements on single leaves, using the non-linear least squares regression procedure NLIN of the statistical software SAS, version 5 (Anon., 1985). The equation is:

$$P_n = -R_d + (P_m + R_d) \times \text{EXP}\left(-\frac{\varepsilon \times H}{P_m + R_d}\right)$$

where: $P_n$ is the net photosynthesis rate (mg $CO_2$ m$^{-2}$ s$^{-1}$),

$P_m$ is the maximum rate of photosynthesis, which is reached at light saturation (mg $CO_2$ m$^{-2}$ s$^{-1}$),

$R_d$ is the respiration rate, measured in the absence of photosynthesis in the dark (mg $CO_2$ m$^{-2}$ s$^{-1}$),

$\varepsilon$ is the initial light use efficiency for fixing $CO_2$ (mg $CO_2$ J$^{-1}$), and

$H$ is the incident flux of photosynthetically active radiation (400–700 nm; W m$^{-2}$).

Treatment differences in single photosynthetic parameters were evaluated in analyses of variance, using Tukey's studentised range procedure for multiple comparisons, as implemented in SAS procedure GLM. Treatment differences with respect to the complete photosynthesis light response curve are analysed in multivariate analyses of variance, as implemented in the SAS ANOVA/MANOVA procedure. Wilks' $\lambda$-statistic was used for significance testing (e.g. Chatfield & Collins, 1980).

## Results

### Aphid populations

In the glasshouse, aphids were present until the 11–13 leaf-stage on 20 June. The maximum

Table 1. *Quantities of different amino-acids (expressed as percentages of the total weight of amino-acids) in honeydew, produced by* Aphis fabae *on sugar beet*

| | Amino-acid | Plant development stage (number of leaves) | | |
|---|---|---|---|---|
| | | 3–4 | 6–8 | 10–12 |
| 1. | Alanine | 0.6 | 0.3 | 0.9 |
| 2. | Arginine | —* | — | 0.2 |
| 3. | Asparagine | 3.8 | 2.6 | 5.6 |
| 4. | Aspartic acid | 23.0 | 30.0 | 13.3 |
| 5. | Butyric acid | — | 0.5 | 0.8 |
| 6. | Cysteine | 0.4 | — | — |
| 7. | Glutamic acid | 17.3 | 43.6 | 10.5 |
| 8. | Glutamine | 45.6 | 16.4 | 20.1 |
| 9. | Glycine | 0.6 | 0.3 | 0.4 |
| 10. | Histidine | — | — | 1.6 |
| 11. | Isoleucine | 0.1 | 0.3 | 4.9 |
| 12. | Leucine | 2.1 | 2.8 | 13.9 |
| 13. | Lysine | — | — | 0.8 |
| 14. | Phenylalanine | — | 0.3 | 5.3 |
| 15. | Proline | 2.7 | 1.5 | 5.3 |
| 16. | Serine | 0.9 | 0.6 | 2.1 |
| 17. | Threonine | 1.0 | 0.2 | 0.8 |
| 18. | Tryptophan | — | — | 2.5 |
| 19. | Tyrosine | 1.6 | 0.5 | 4.8 |
| 20. | Valine | 0.3 | 0.2 | 6.3 |
| | % amino-acids of dry matter | 15.6 | 10.6 | 3.6 |

* not detected

population density occurred at the 9–10 leaf stage on 14 June: about 3000 aphids per plant. In the field, the population reached a maximum of slightly over 2000 aphids per plant at the 9–12 leaf-stage at 23 June. The numbers subsequently declined rapidly.

### Composition of honeydew

The amino-acid composition of honeydew produced by *A. fabae* is shown in Table 1. There was a marked decline in the amino-acid content as the plants grew: at the 3–4 leaf stage, the honeydew contained 15.6% amino-acids in the dry matter, while at the 10–12 leaf stage, this percentage had diminished to 3.6%. Many different amino-acids were detected: 14, 15 and 19 in the three samples and 20 amino-acids altogether. In the first two samples, aspartic acid, glutamic acid and glutamine were present in the largest amounts, making up 86% and 90%, respectively, of the total amino-acid dry matter. At the 10–12 leaf stage, these three amino-acids made up 44% of the amino-acid dry matter, while leucine and several other amino-acids occurred in larger relative amounts than before. Artificial honeydew was prepared three times according to the compositions given in Table 1. Each solution was used two times for spraying the plants (see Materials and Methods).

### Amount of artificial honeydew applied

Fig. 1 shows estimated trends of the sugar concentration on leaves of untreated, aphid-infested and honeydew-sprayed plants. The sugar concentration on leaves of aphid-infested plants varied in a narrow range, 0.17 and 0.22 mg cm$^{-2}$, in five sample occasions, consistently higher than the 0.01 to 0.06 mg cm$^{-2}$ that was found on the control leaves. Plants sprayed with artificial honeydew showed greater temporal variation in leaf surface sugar concentration than the other two curves, with consistent steep increases after honeydew
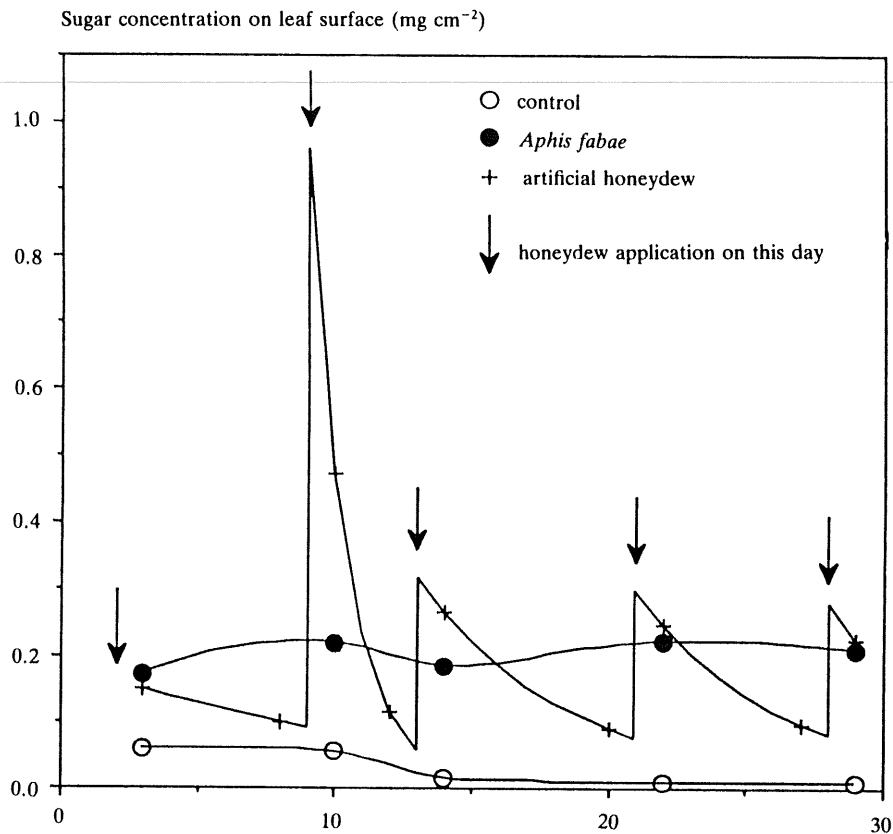
Sugar concentration on leaf surface (mg cm$^{-2}$)



Fig. 1. Time-course of sugar concentration on leaf surfaces in field grown sugar beet. Control (O); *Aphis fabae* (●), Artificial honeydew (+). In the honeydew treatment, the concentration reaches an abrupt peak at the date of application. The crosses are observed data. The peaked drawn line assumes an exponential decay of sugars on the leaf surface between honeydew application dates.

applications. The trend drawn in Fig. 1 assumes an exponential decay of sugar between applications, which is doubtless a simplification of reality. Many different processes, such as rainfall, microbial sugar consumption and leaf expansion, are likely to be involved in the decrease of leaf surface sugar in the honeydew treatment between application dates. The figure suggests that the artificial sprays produced an integral of sugar concentration on the leaf surface over time that was similar to that occurring on the aphid-infested leaves, which was what we tried to achieve.

### Density of stomata and honeydew coverage

The density of stomata was about 43 mm$^{-2}$ on the upper leaf surface and 50 mm$^{-2}$ on the lower. Hardly any honeydew was found on the lower leaf surfaces. On the upper surfaces, the percentage of stomata coverage by natural honeydew was 33.3 ± 4.4 (s.e.m), with single honeydew plaques covering in the order of magnitude of 5 to 10 stomata (Fig. 2). The percentage of stomata covered by artificial honeydew was quite similar: 33.0 ± 6.5. This similar coverage was obtained with a somewhat larger number of plaques of smaller size (not further quantified).
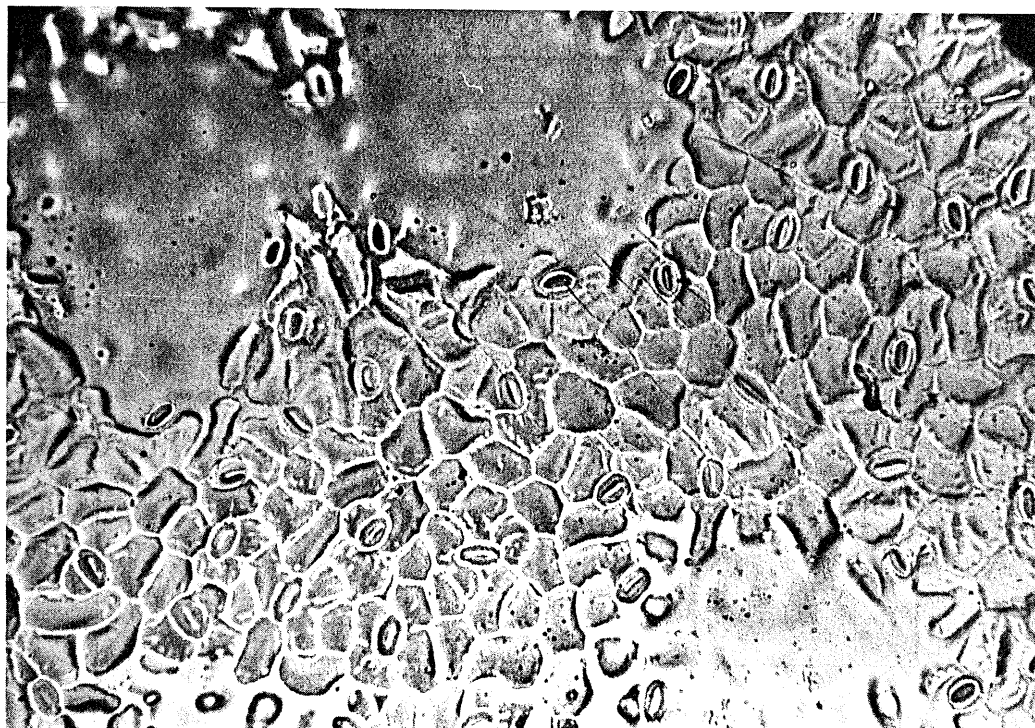
Fig. 2. Micro-photograph (190×; 1 cm = 50 $\mu$m) of silicone rubber imprint of a sugar beet leaf, covered with natural honeydew.

### Aphid feeding, honeydew and photosynthesis

The photosynthesis measurements of individual leaves at different light intensities confirmed quite well to the description provided by the negative exponential equation (Fig. 3). $R^2$ values averaged 0.99, ranging from 0.974 to 1.00 (10% and 90% percentile, respectively), while the root of the residual variance averaged 0.04 mg $CO_2$ m$^{-2}$ s$^{-1}$. The data were further analysed by performing univariate (ANOVA) and multivariate analysis of variance (MANOVA) on the parameters of the fitted curves. A similar methodology was used by Keuls & Garretsen (1982). The results are presented in Tables 2, 3 and 4, which show the treatment, leaf age and (for Table 2) the measurement date marginal means calculated by the factorial analysis. ANOVA showed that dark respiration ($R_d$), light use efficiency ($\varepsilon$) and maximum rate of photosynthesis ($P_m$) were all not significantly affected by *Aphis fabae* or artificial honeydew (Tables 2, 3 and 4), even though the most severely-infected leaves and those most heavily-covered with artificial honeydew were selected for measurements. The lack of treatment effects is very consistent (Tables 2, 3 and 4), and the treatment differences that occur are much smaller than the minimum significant differences ($\alpha = 0.05$). The multivariate comparison of the complete photosynthesis light response curves confirmed this lack of treatment effects.

The measurements did demonstrate that the younger leaves had higher rates of dark respiration, possibly due to energy demand of growth and synthesis processes. Younger leaves also exhibited a lower maximum rate of photosynthesis and a lower light use efficiency than older leaves (Tables 2–4). The light use efficiency differed strongly from one measuring date to another. These differences did not correlate with differences among dates in relative
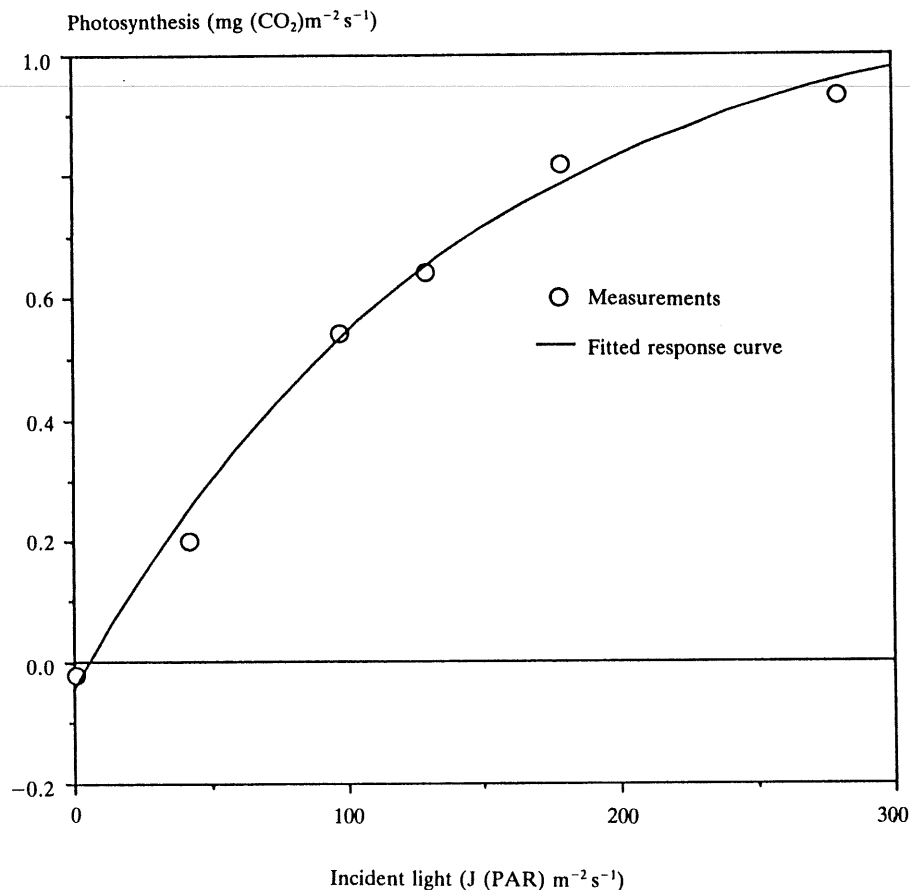
Photosynthesis (mg $(CO_2)m^{-2}s^{-1}$)



Incident light (J (PAR) $m^{-2}s^{-1}$)

Fig. 3. Measured $CO_2$ exchange rates (O) and fitted photosynthesis light response curve of aged *Aphis fabae*-infested sugar beet leaf (leaf number 5) in the glasshouse on 24 June. Parameter values: $P_m = 1.12 \pm 0.11$ mg $CO_2$ $m^{-2}s^{-1}$; $R_d = 0.051 \pm 0.039$ mg $CO_2$ $m^{-2}s^{-1}$; $\varepsilon = 8.2 \pm 1.1$ $\mu$g $CO_2$ $J^{-1}$.

humidity, vapour pressure deficit, temperature or radiation on or before the measuring date. The multivariate analysis of the gas exchange parameters confirmed the highly significant influences of leaf age and measuring date.

## Discussion

In this study we did not discover effects of *Aphis fabae* or its honeydew on the photosynthesis of sugar beet leaves. It is possible that real differences were overshadowed by measurement error and biological variation, but this explanation seems implausible as honeydew-sprayed plants had the same rate of growth as unsprayed plants (Hurej & van der Werf, 1993). The absence of a honeydew-effect may be related to the incomplete coverage of the stomata on the upper surface of the leaf, 33%, while the stomata on the lower surface remained almost honeydew-free. Therefore at least 80% of the stomata were still functional, which may be sufficient for normal rates of photosynthesis. Furthermore, no pathogenic or saprophytic fungi developed on the honeydew. So possible shading or

Table 2. *Effect of* Aphis fabae *and artificial honeydew on* $CO_2$ *exchange rates of sugar beet plants in the glasshouse, measured with ADC portable equipment*

|  | $R_d$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $P_m$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $\varepsilon$ (mg $CO_2$ J$^{-1}$) |
|---|---|---|---|
| *Treatment means* |  |  |  |
| Aphis fabae | 0.09 a[1] | 0.92 a | 7.9 a |
| Control | 0.08 a | 0.88 a | 7.2 a |
| Honeydew | 0.09 a | 0.87 a | 7.3 a |
| L.S.D. (0.05)[2] | 0.03 | 0.18 | 1.2 |
| M.S.D. (0.05)[3] | 0.03 | 0.22 | 1.4 |
| MANOVA: | Wilks' $\Lambda_{3,2,28} = 0.845$ (N.S.)[4] |  |  |
| *Leaf age means* |  |  |  |
| Old | 0.07 a | 0.96 a | 8.4 a |
| Mature | 0.08 a | 0.98 a | 7.4 ab |
| Young | 0.12 b | 0.73 b | 6.6 b |
| L.S.D. (0.05) | 0.03 | 0.18 | 1.2 |
| M.S.D. (0.05) | 0.03 | 0.22 | 1.4 |
| MANOVA: | Wilks' $\Lambda_{3,2,28} = 0.258$ ($P < 0.0001$) |  |  |
| *Measuring date means* |  |  |  |
| 03.06 | 0.10 a | 0.92 a | 10.1 a |
| 10.06 | 0.06 b | 0.78 a | 5.7 b |
| 18.06 | 0.09 ab | 1.00 a | 6.5 bc |
| 24.06 | 0.10 ab | 0.86 a | 7.6 bc |
| L.S.D. (0.05) | 0.03 | 0.21 | 1.4 |
| M.S.D. (0.05) | 0.04 | 0.28 | 1.8 |
| MANOVA: | Wilks' $\Lambda_{3,3,28} = 0.198$ ($P < 0.0001$) |  |  |

Number of leaves evaluated: 36.

[1] Means significantly different in Tukeys studentised range test for multiple comparisons at $\alpha = 0.05$ have different lettering.

[2] L.S.D. = least significant difference in $t$-test for single comparisons at $\alpha = 0.05$.

[3] M.S.D. = minimum significant difference in Tukeys studentised range test for multiple comparisons at $\alpha = 0.05$.

[4] Wilks' $\Lambda_{p,h,e}$ statistic has parameters $p$ = number of variables, $h$ = hypothesis degrees of freedom and $e$ = error degrees of freedom.

pathological effects of these fungi did not occur. The lack of honeydew effects on sugar beet photosynthesis conflicts with current opinion (Jones & Dunning, 1972; Cammell, 1981), which is however based on experiments on other plant species than sugar beet. Leaves with black bean aphids had, in our measurements, the same gas exchange parameters as control leaves. This finding confirms the results and hypotheses of Hawkins *et al.* (1987) who found increased net photosynthesis rates in aphid-infested broad bean and cowpea plants, and suggested that the aphids caused an uprising in photosynthesis that compensated for their assimilate intake and respiration.

In several other interactions between plant species and homopteran (sucking) insects, the honeydew produced has detrimental effects on leaf functioning or longevity (Rabbinge *et al.*, 1981; Fokkema *et al.*, 1983; Wood *et al.*, 1985). Rabbinge *et al.* (1981) found that coverage of 33% of the stomata of wheat by honeydew negatively affected the photosynthesis. Aphid infestation in wheat occurs at the end-phase of growth when the leaves senesce within a few weeks. Honeydew accelerates senescence in this situation (Vereijken, 1979; Rabbinge *et al.*, 1981) and hence the associated decline of the maximum rate of photosynthesis is advanced. *Aphis fabae* occurs during the exponential growth phase of the

Table 3. *Effect of* Aphis fabae *and artificial honeydew on $CO_2$ exchange rates of sugar beet plants in the field, measured with ADC portable equipment*

| | $R_d$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $P_m$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $\varepsilon$ (mg $CO_2$ J$^{-1}$) |
|---|---|---|---|
| *Treatment means* | | | |
| Aphis fabae | 0.08 a | 0.85 a | 5.7 a |
| Control | 0.07 a | 0.91 a | 5.3 a |
| Honeydew | 0.06 a | 0.85 a | 5.7 a |
| L.S.D. (0.05) | 0.02 | 0.17 | 1.7 |
| M.S.D. (0.05) | 0.03 | 0.21 | 2.1 |
| MANOVA: | Wilks' $\Lambda_{3,2,6} = 0.463$ (N.S.) | | |
| *Leaf age means* | | | |
| Old | 0.08 a | 1.07 a | 5.7 a |
| Mature | 0.07 a | 0.93 a | 5.7 a |
| Young | 0.05 a | 0.56 b | 5.3 a |
| L.S.D. (0.05) | 0.02 | 0.20 | 1.9 |
| M.S.D. (0.05) | 0.03 | 0.28 | 2.7 |
| MANOVA: | Wilks' $\Lambda_{3,3,6} = 0.036$ ($P = 0.04$) · | | |

Number of leaves evaluated: 12 (three young, six newly mature, three fully mature).

sugar beet crop, May to June, when the leaves have a long expected life span (Milford, Pocock, Riley & Messem, 1985). This may explain why honeydew had no measurable consequences in our study. The results of our work refute honeydew as one of the mechanisms listed by Cammell (1981) as causes of damage by black bean aphid in sugar beet, at least when no fungi develop on the honeydew.

As effects of *Aphis fabae* or honeydew were not detected in this study, while in the same experiment, severe growth reductions were recorded (Hurej & van der Werf, 1993), the main mechanism of injury is likely to be assimilate withdrawal (Cammell, 1981). Assimilate drain during the early growth stages may severely reduce the relative growth rate of early colonised plants (Groenendijk, van der Werf, van Dijk & Carneiro, 1990) and postpone the development of full leaf cover by the crop, having repercussions for yield.

Table 4. *Effect of* Aphis fabae *and artificial honeydew on $CO_2$ exchange rates of sugar beet plants in the glasshouse, measured with a laboratory setup*

| | $R_d$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $P_m$ (mg $CO_2$ m$^{-2}$ s$^{-1}$) | $\varepsilon$ (mg $CO_2$ J$^{-1}$) |
|---|---|---|---|
| *Treatment means* | | | |
| Aphis fabae | 0.06 a | 0.76 a | 14.2 a |
| Control | 0.06 a | 0.73 a | 12.3 a |
| Honeydew | 0.06 a | 0.78 a | 13.2 a |
| L.S.D. (0.05) | 0.05 | 0.22 | 13.1 |
| M.S.D. (0.05) | 0.06 | 0.28 | 17.2 |
| *Leaf age means* | | | |
| Old | 0.06 a | 0.85 a | 15.4 a |
| Mature | 0.06 a | 0.66 b | 11.0 a |
| L.S.D. (0.05) | 0.04 | 0.18 | 10.7 |
| M.S.D. (0.05) | 0.04 | 0.17 | 10.2 |

Number of leaves evaluated: 6
No MANOVA was performed because of insufficient observations.

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