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Role of nitrogen content of pea (*Pisum sativum* L.) on pea aphid (*Acyrthosiphon pisum* Harris) establishment

Gh.H. Moravvej* and S. Hatefi

Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashad, P.O.Box 1163, Mashhad, Iran
**Corresponding author's E-mail: Moravej@ferdowsi.um.ac.ir*

ABSTRACT
The leaf nitrogen content is generally accepted as an indicator of food quality and as a factor affecting host selection by phytophagous insects. The alate pea aphids (*Acyrthosiphon pisum* Harris, Aphididae) were given a choice among non-nodulated pea plants (*Pisum sativum* L.) supplied with one of four nitrate-N levels (0, 3, 15 and 30 mM). When whole plants were exposed to aphids for 7 days, the results indicated that the settling response of alatae, and subsequently the reproduction of alighted aphids, increased as the level of N supply or the concentration of total soluble nitrogen of the leaves increased, with the exception of the highest N supply (30 mM N). However, the density of settled alatae (in terms of number per unit leaf area) increased as the level of N supply decreased, being greatest on N-deficient plants (0 mM N treatment) and lowest density on N-sufficient (15 mM N) and N-excess (30 mM N) plants. In a free-choice experiment, equal-sized leaf discs taken from the different N treatments were exposed to alate adults for 24 h.The settling response of aphids was positively affected by leaf colour (yellowing), with the greatest number settled on yellow leaf discs (N-deficient plants) and fewest settled on green or dark-green discs (N-sufficient and N-excess plants). Relationships between level of N supply, total soluble nitrogen concentration, total chlorophyll concentration, plant growth parameters and aphid abundance (number of alatae per plant) or density (number of alatae per unit leaf area, or per leaf disc) were established. The implications of results for integrated aphid management were discussed.

Keywords: Aphididae, chlorophyll, host selection, pea aphid, soluble nitrogen.

INTRODUCTION
Nitrogen is frequently considered as a limiting resource for insects (for reviews: Mattson 1982; Bernays 1992). The leaf nitrogen content is generally accepted as an indicator of food quality (Scriber & Slansky 1981) and as a factor affecting host selection by phytophagous insects (McNeill & Southwood 1978; Mattson 1982; Bernays 1992). Chemical fertilizers are able to modify the nutritive value of plants for phytophagous insects and affect their establishment and population growth (Van Emden 1966; McClure 1980; Prestidge 1982; Jansson & Smilowitz 1986; Minkenberg & Fredrix 1989; Dowell & Steinberg 1990; Jauset *et al*., 1998, 2000).

The importance of "environmentally induced plant resistance" to aphids using treatments such as fertilizers has been emphasized in aphid pest management (Van Emden 1987; 1990; 1997). These studies suggest that the variation in levels of resistance to aphids induced by fertilisation treatments in plants of a single variety might be greater than those between nominally 'susceptible' and 'resistant' varieties. However, the extent to which nitrogen can be manipulated to benefit integrated pest management is not well understood. Comparing population parameters of *Myzus persicae* (Sulzer) on potatoes fertilized with different levels of nitrogen (N) in a field study, Jansson & Smilowitz (1986) showed that abundance of aphids was not

significantly different among N treatments, although the trends implied that it was greatest in the intermediate N level and least on the highest or lowest N levels. However, in the same study, it was demonstrated that the population growth rate of *M. persicae* increased significantly as the level of N applied increased, with the exception of the highest N level (which reduced the population growth rate of aphids). Jansson & Smilowitz's (1986) study did not assess the preference of winged aphids (alatae) for the plants, because aphid counts were made for all stages and morphs. The lower population density of aphids on a resistant plant (either genetically or induced by physiological treatments such as fertilizers) is the result of either a lower initial infestation by winged migrants ("non-preference" or "antixenosis" when genetically based antixenotic resistance is concerned), and/or adverse effects of plants on the aphids (antibiosis) following infestation (Painter 1951; Cartier 1963).

A survey of literature did not show any experimental evidence whether settling by alate aphids was affected by fertilizer treatments. Most studies have been concerned with growth, reproduction and survival of non-winged individuals (apterae) feeding on different plant varieties. These results may not be relevant to agricultural areas where plants of in-bred lines would be colonized initially and primarily by winged individuals (alatae). In field conditions, alate aphids have little control over the direction of their flight because of their low flight speed relative to that of air movement. They are displaced by atmospheric turbulence (Taylor 1965; Van Emden 1972). However, once within the layer of relatively still air around vegetation, called the "boundary layer", aphids can control their landing on plants (Taylor 1960, 1974; Kennedy 1976; Dixon 1998). Near to vegetation, it has been suggested that the settling response of alate aphids is far more due to visual than olfactory stimuli (Kennedy & Stroyan 1959; Van Emden 1972; Dixon 1998). After settling, an aphid recognizes a potential host by the structure and chemistry of its surface and internal tissues (Dixon 1998). The physiological and ecological interactions involved in host-plant location and recognition by aphids have been reviewed and discussed by Dixon (1998). The effects of nutritional quality of the plant on alighting and colonising by alate aphids have frequently been noted through comparison of different plant species, or different varieties, but there is no data for such comparisons between fertilized and unfertilised plants of a single variety. In a field trial with several pea varieties, Cartier (1963) demonstrated that the alighting response of winged migrants of *A. pisum* was associated with the foliage colour of varieties at early seedling stage and with plant height at the later stages. As variations in leaf colour or plant size might be produced by N-fertilisers, it is expected that N treatments will affect the settling preferences and colonisation by alate aphids.

The pea aphid (*A. pisum*)-pea (*Pisum sativum* L.) system was used in this study, under controlled laboratory conditions. The aim was to assess how the settling preference of alate aphids and their subsequent nymphiposition might be influenced by nitrogen content of the host plants. The implications of the results are discussed with respect to the use of physiological treatments such as fertilizers in integrated aphid management.

MATERIAL AND METHODS

Growth condition of plants and aphids

All experimental plants were maintained in a controlled temperature (CT) room with a L16-D8 cycle, 23-18 °C day-night temperature, 60-80% relative humidity and photosynthetic photon flux density (400-700 nm) of 80-130 and 300-550 μmol m^{-2} s^{-1} measured at the surface and 50-cm above the bench respectively. All the test plants and aphid cultures were kept in the same CT room in 75×50×50 cm-screen wooden cages. The four sides of the each cage were covered by screen cloth, and the top by clear acrylic sheet (PERSPEX sheet, Ineos Acrylics UK Ltd, Hampshire, UK).

Plant culture and nitrogen nutrient solutions

Seeds of *Pisum sativum* L., var. Meteor (Sharpes International Seeds Limited, Sleaford, UK) were surface-sterilised (for 30 seconds) by immersion in 6% (v/v) hypochlorite, soaked in water overnight and germinated in vermiculite (Dupré Vermiculite, Hertford, UK; Grade DSF, 0.5-1.0 mm particle size) in an incubator (26 °C). After 3 days, 60-95% of seeds germinated. To

prevent nodulation in plants (and so to allow growing only under NO3-N treatments), all pots and saucers were surface-sterilised by immersion for 1 h in 10% hypochlorite and the bottom of the plant cages was washed with this disinfectant. Two-day old seedlings (usually between the second small scale leaf and first node stage, 101) (Knott 1987), selected for size uniformity, were transferred individually into 10 cm diameter plastic pots containing washed fine sand, which had been saturated with the respective nutrient solution.

The nutrient solution was a modified formulation based on Rorison nutrient solution (Rorison & Robinson 1986). It was prepared in distilled water with the following base composition: $Ca(NO_3)_2.4H_2O$ (concentration varied depending on treatment concerned), $CaCl_2$ (0.5 mM), $MgSO_4.7H_2O$ (1mM), KH_2PO_4 (1 mM), K_2HPO_4 (0.3 mM), K_2SO_4 (0.45 mM) Fe-EDTA (0.0681 mM) and trace-element solution (1 ml per litre). The trace-element solution was composed of $Na_2MoO_4.2H_2O$ (0.0001 mM), $MnCl_2.4H_2O$ (0.009 mM), H_3BO_3 (0.0463 mM), $ZnSO_4.7H_2O$ (0.0008 mM) and $CuSO_4.5H_2O$ (0.0003 mM). Using $Ca(NO_3)_2.4H_2O$, four different nitrogen (NO_3-N) treatments, 0, 3, 15 and 30 mM N, were prepared. The changes in calcium concentration were corrected using $CaCl_2$. The plants were watered daily with nutrient solution; 25 ml in the first week, 50 ml in second week, 75 ml in the third week and 100 ml per pot thereafter. The drained solution was discarded. The nutrient solution treatments continued until the conclusion of the experiments.

Aphid culture and production of adult alatae

A clone of anholocyclic *A. pisum* was established on greenhouse-grown broad beans (*Vicia faba* L. var. *major* Imperial White Windsor), by a single parthenogenetic apterous aphid taken from a long-established anholocyclic aphid colony. The aphid stock culture was maintained on 2-3-week old plants grown in potting compost watered every other day with tap water, and kept in 75×50×50 cm screen cages in the CT room under the same conditions as the experimental plant culture.

About one week before adult alatae were needed for preference tests, the regular weekly renewing of plants in aphid stock culture was not done; this led to crowded aphid population and so production of alate virginoparae (hereafter referred to alatae or alate adults). Preliminary experiments showed that using alate aphids of different ages caused high variation in the preference tests. Therefore, a large number of late instar aphids (normally third instar nymphs and fourth instar alate nymphs) were selected from the crowded culture, and transferred onto stipules (young broad been seedlings with the growing tip removed) again in crowded conditions. Daily culture of these alatae ensured a regular supply of newly moulted adults for preference experiments. Post-teneral_aphids making their first flight gathered on the roof and walls of the cage. When they were 1-3 day old, they were collected and used for the experiments.

Settling preference experiments

Pea plants (*P. sativum*) supplied with the four N treatments were used when they reached the beginning of the reproductive stage (Enclosed bud stage, code 201), being chronologically 21-day old. Plants supplied with N-free solution (0 mM N) had normally one or two nodes less (5-6 nodes) than those supplied with other N solutions (i.e., 3, 15 and 30 mM N). Counts of nodes were based on those having fully-expanded leaves, so excluded the first two nodes on the bottom of the plant containing small scale leaves and the last node (with folded leaf) on the top (Knott 1987).

Two free-choice experiments were conducted on different occasions. The design of the second experiment was based on the hypothesis derived in the first one (see results and discussion). Separate series of plants, grown in the same conditions, were prepared for the two experiments.

Whole plant (Experiment 1)

Adult alatae were given a choice among plants grown with the four N levels in a randomised complete block design; the 75×50×50-cm cages were used as testing arenas and considered as blocks, and five such cages were used. The four test plants (each supplied with one of the N levels) were arranged randomly and equidistantly within each cage. Sixty adult alatae were introduced using glass vials placed centrally into each

cage, on a stand as high as the plant pot surface. Preference was determined based on the number of alatae settled on each plant, recorded at two times, 24 h and 7 days after release. At 24 h, records were also made on numbers of alatae settled on different plant parts (including adaxial and abaxial surfaces of stipules and leaflets, tendril, stem, bud, flower and shoot terminal). The shoot terminal was considered to consist of the top-most internode plus an unexpanded leaf.

The total number of progeny deposited by alatae was recorded on each plant after 7 days. This was done before the earliest deposited nymphs reached reproductive maturity (by this time, a few nymphs had developed to apterous adults, but they had not commenced reproduction). The number of nymphs deposited per plant was corrected for alatae recorded at the 7th day and used as an additional dependent variable.

At the end of experiment, i.e., when plants were 28-day old, the height of shoot, total stipules area, total leaflet area and the total leaf area (i.e., sum of stipules and leaflet areas) were determined. From the latter data and records of alatae at the 7th day, the number of alatae per unit leaf area (i.e., aphid density) was calculated for each plant replicate.

Leaf disc (Experiment 2)

To test the hypothesis that settling behaviour of *A. pisum* alatae is affected by the colour of the substrate, aphids were given a choice among equal-sized leaf areas of plants supplied with the four N treatments, which were considered to vary in colour. The colour of the leaves was quantified by measuring total chlorophyll content (the method described in the next section). Leaf discs (1 cm diameter) were cut from medium-aged leaves (positioned on the 4th-6th nodes which varied depending on N treatment) of the 21-day old plants of the respective N treatments. Two leaf discs from each of the four test plants (N treatments) were randomly arranged equidistantly in a 9 × 1.5 cm plastic Petri-dish (considered as a block) with moist filter paper in the base. The Petri-dish (with the lid removed) was covered using a funnel to provide more space in the arena. Twenty adult alatae were released into the arena from the top then it was closed by a piece of cotton wool (Figure 1). There were six such testing arenas set up on the bench of the CT room in a randomised complete block design. After 24 h, the leaf discs were examined for alatae, by which time most had settled. The average number of aphids settled on the two leaf discs of each N treatment for each arena was used as a datum for statistical analysis after appropriate transformation.

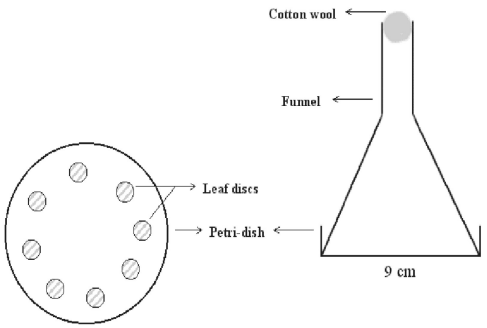


Fig 1. Set-up for preference test of alate aphids on leaf discs; large circle (left) shows moist filter paper on the base of Petri-dish; leaf discs (taken from plants of the respective N treatments) were numbered at appropriate position on the external margin of Petri-dish wall.

Method for total soluble nitrogen analysis

A replicate set of aphid-free 21-day old plants of the four N treatments were prepared, and their medium-aged leaves (on nodes 3-6) were dried using a freeze-dryer. The leaves of 5-10 plants were pooled within the respective N treatments, to provide sufficient materials for analysis, being considered as a sample. Three such samples per treatment were used. The samples were ground to a fine powder and 500 mg of each was extracted with de-ionised water containning 2.5% trichloroacetic acid in 0.02% phenol on an automatic shaker for 3 hours. The extract was heated in a water bath (100 ^{o}C) for ten minutes, and then centrifuged at 2500 rpm for 30 minutes. The clear supernatant was transferred to a round-bottom (Kjeldahl) flask, and then dried using a freeze-dryer. The semi-micro Kjeldahl procedure was used for determination of total soluble nitrogen (Allen 1989), briefly described as follows. Each sample (i.e., the dried residue in a flask) was digested with 5 ml concentrated H_2SO_4 and one (2.5 g) Kjeldahl tablet (containing 20 parts $CuSO_4$, 0.05 parts Selenium and 100 parts Na_2SO_4) for 2 hours. After cooling, the digest was diluted to 50 ml with de-ionised water.

The digestion stage converts organic nitrogen to ammonium–nitrogen. The micro-diffusion technique (Conway 1962) and titration were used for liberating and estimating ammonium-nitrogen. The ammonia was liberated from the diluted digest after addition of NaOH (40%) using a distillation apparatus (Buchi 321 distillation unit). Prior to running the samples, the distillation apparatus was checked by a recovery test with NH_4Cl as nitrogen standard. The distillate was collected into 10 ml of 2% boric acid with an indicator (125 mg methyl red and 82.5 mg methylene blue in 100 ml of 90% ethanol), and titrated with M/140 HCL. The soluble nitrogen results were expressed as percent dry weight.

Method for leaf pigment measurement

It has been generally regarded that leaf colour is highly correlated with nitrogen content (Raese 1977; Pfeiffer & Burts 1984). This has been attributed to the number and size of chloroplasts, both of which decrease as N supply decreases (Kutik *et al*., 1995; Lawlor *et al*., 2001), leading to leaf senescence and chlorosis (yellowing) (Brouquisse *et al*., 2001). In the present study, total chlorophyll concentration of the leaf was used as a quantitative criterion for leaf colour. Measurements were done based on the method of Wellburn (1994), whereby the leaf pigments including individual chlorophylls *a* and *b*, as well as total carotenoids were determined spectrophotometrically in leaf extract. Acetone 80% was used as a solvent. Three samples per N treatment were used. A sample consisted of six leaf discs (each 1-cm diameter) taken from medium-aged leaves (on the 4^{th}-6^{th} nodes) of one plant. The plants for this purpose were the same age as, and grown concurrently with the plants of Experiment 2. Leaf discs were ground in 2 ml acetone (80%) using a pestle and mortar. The extract was transferred to a graduated tube and made up to a total volume of 10 ml with acetone (80%), assayed immediately using a spectrophotometer (Jenway Model 6405, Jenway Limited, Essex, UK). The absorptions for each sample were read at the wavelengths 663 nm, 646 nm and 470 nm. The concentrations of the two chlorophylls (C_a and C_b) and of total carotenoids (C_{x+c}) as µg per ml extract were calculated using the following equations established by Lichtenthaler and Wellburn (1983):

$C_a = 12.21A_{663} - 2.81A_{646}$

$C_b = 20.131A_{646} - 5.03A_{663}$

$C_{x+c} = (1000A_{470} - 3.27C_a - 104C_b)/198$

In these equations, the letter *A* refers to the absorption at the wavelength specified in the subscript. Concentrations of these pigments were then expressed as µg per cm^2 leaf area. The sum of chlorophylls *a* and *b* provided the total chlorophylls, being used as a leaf colour scale.

Experimental design and statistical analyses

The experimental layout for each experiment (aphid preference experiments, soluble nitrogen and pigments analyses) was a randomised complete block design, block (cage, Petri-dish or group of samples) being regarded as a random factor, and N treatment as a fixed factor. Data were subjected to analysis of variance (ANOVA) using "Univariate" option of GLM within SPSS computer programme (SPSS 1998). However, as analyses for all dependent variables indicated no significant effect of block, hence only the results of one-way ANOVA are reported, followed by Duncan's multiple range tests to separate treatment means. Before performing analyses of variance, in order to stabilise variance and/ or improve normality, alatae counts per plant, per plant part or per unit (cm^2) leaf area (Experiment 1), or per leaf disc (Experiment 2) were transformed by LN (n+1), and nymphs counts per plant or per alata (Experiment 1) by LN (n).

Relationships between certain pairs of plant variable, aphid variable, total soluble N concentration or the level of N supply were tested using linear and/or polynomial quadratic regression models (explained in results). The relationship between treatment mean of the number of the alatae per leaf disc and leaf colour scale was examined using Spearman's rank-order correlation.

RESULTS

Response of pea plants to NO_3-N fertilisation

Growth of pea plants was significantly affected by levels of nitrate-N of nutrient solution, as judged by shoot height ($F_{3, 16}$ =

52.5, P<0.001), total stipule area ($F_{3, 16}$ = 92.3, P<0.001), total leaflet area ($F_{3, 16}$ = 88.1, P<0.001) or total leaf (leaflet plus stipule) area ($F_{3, 16}$ = 102.3, P<0.001). All growth components increased as N level increased up to 15 mM N, but then decreased by 30 mM N (Figure 2). Relationships between the growth components (Y) and N level of nutrient solution (X) were quantified better by quadratic polynomial regressions than by linear models, as shown by higher coefficients of determination (R^2) and higher significance levels of the former model (Table 1); nevertheless all these relationships should be considered with caution, as only four N rates were used.

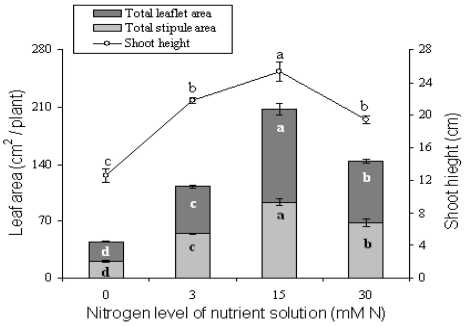


Fig 2. Growth components (mean ± s.e., n=5), expressed as shoot height, and total stipule and leaflet areas, of 28-day old pea plants (*P. sativum*, var. Meteor) grown with different nitrogen concentrations (as nitrate in nutrient solution); within a component means with by the same letter do not differ significantly (P>0.05, Duncan's multiple range test).

Plants supplied with N-free solution (0 mM N) showed symptoms of severe nitrogen deficiency. The symptoms were observable on 7-14 day old plant as light green leaves and gradually progressed so that at the end of experiments (28-day old plants) the older leaves (on 1st and 2nd nodes) were dried and other leaves were chlorotic (yellow). At the end of each experiment (i.e., 28-day old plants), the plants supplied with 30 mM N exhibited symptoms of nitrate toxicity (as necrotic lesions on leaf surface) on the older leaves (1st or 2nd nodes). Plants supplied with 3 mM N showed slight nitrogen deficiency, as observed by pale green colour of all leaves except the 2-3 normal (green) leaves on the top most part of plant. The level of 15 mM N resulted in vigorous plants without any symptom of N-deficiency or –toxicity.

Relationship between total soluble nitrogen or leaf pigments, and N treatments

Increase in the supply of N significantly increased total soluble N concentration of the leaves ($F_{3, 8}$ = 28.68, P<0.001). However, Duncan's multiple-range test indicated that the difference in soluble N of the leaves was not significant between the two highest N supplies (i.e., 15 and 30 mM) (Figure 3). Regressing total soluble N (y) on N levels in nutrient solution (x) indicated that 62% of variation in soluble N content of leaves was explained by the levels of N supply, if judged by a linear model ($y = 1.49 + 0.058x$, $F_{1, 10}$ = 16.39, R^2 = 0.621, P<0.01). The amount of variability explained increased to 80%, when a polynomial quadratic regression was used ($y = 1.17 + 0.181x - 0.004x^2$, $F_{2, 9}$ = 18.61, R^2 = 0.805, P<0.001).

Table 1. Relationships between growth component (Y) of pea plant (*P. sativum*) and the level of nitrate-N fertilisation (X), expressed by linear and polynomial quadratic regressions (n=20 for all).

Dependent variable (Y)	Regression model	Significance level	R^2
Shoot height	$Y = 18.1 + 0.14X$	=0.136	0.119
	$Y = 14.9 + 1.40X - 0.04X^2$	<0.001	0.737
Total stipule area	$Y = 42.6 + 1.37X$	<0.01	0.361
	$Y = 25.7 + 7.93X - 0.22X^2$	<0.001	0.915
Total leaflet area	$Y = 48.7 + 1.61X$	<0.01	0.321
	$Y = 26.5 + 10.23X - 0.29X^2$	<0.001	0.939
Total leaf area	$Y = 91.3 + 2.98X$	<0.01	0.342
	$Y = 52.1 + 18.17X - 0.50X^2$	<0.001	0.938

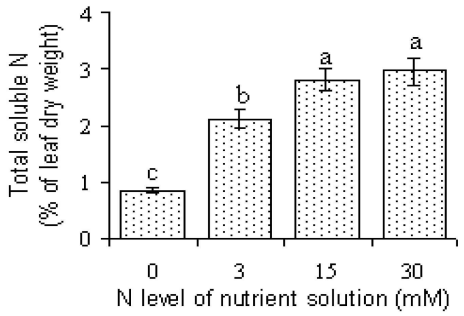


Fig 3. Mean percent total soluble nitrogen concentration in the medium-aged leaves of pea plants (*P. sativum*, var. Meteor) supplied with different concentrations of nitrate-N (0, 3, 15 and 30 mM); bars represent standard errors (n=3).

The contents of all forms of pigments (Chlorophyll *a*, Chlorophyll *b* and total carotenoids) of leaves were significantly affected by the level of N supply (P<0.001 for all), and generally increased as N increased (Figure 4).

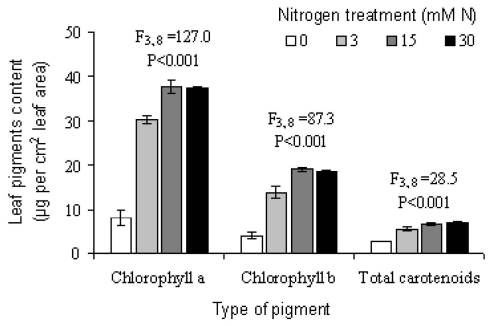


Fig 4. Mean concentration of chlorophyll *a* and *b*, and toal carotenoids, expressed as µg per cm² leaf area, of the medium-aged leaves of pea plant (*P. sativum*, var. Meteor) supplied with different concentrations of nitrate-N (0, 3, 15 and 30 mM); one-way ANOVA results for each variable are presented; bars represent standard errors (n=3).

However, there were no significant differences between the two highest N levels. The typical symptoms of nitrogen deficiency (i.e., leaf chlorosis) is usually attributed to the loss of chlorophylls, thus in this study, the sum of chlorophyll *a* and *b* (total chlorophyll) was considered as a leaf colour scale. Regressing total chlorophylls (y) on N levels in nutrient solution (x) indicated that 54% of variation in total chlorophyll content of leaves was explained by the levels of N supply, if judged by a linear model ($y = 28.59 + 1.13x$, $F_{1, 10} = 11.66$, $R^2 = 0.538$, $P<0.01$). The amount of variability explained increased to 81%, when a polynomial quadratic regression was used ($y = 20.53 + 4.26x - 0.104x^2$, $F_{2, 9} = 19.26$, $R^2 = 0.811$, $P<0.001$). The low chlorophyll contents were associated with the strongly chlorotic leaves (yellowing), the large values with green or dark green leaves, and the intermediate levels of chlorophyll with pale green ones. Mean total chlorophyll contents in plants of the four N treatments are presented in Figure 10.

Settling preference of alatae on whole plants

N treatments significantly influenced the number of adult alatae settled on whole plants, at both recording times (T) (after 24 h: $F_{3, 16} = 17.74$, after 7 days: $F_{3, 16} = 24.61$; $P<0.001$ for both). Analysis by two-way ANOVA (N treatment and recording time (T) as independent factors) for number of alatae per plant revealed that the effects of N treatment on aphid alighting did not differ between the two recording times, as presented by the lack of significant interaction ($N \times T$ interaction: $F_{3, 32} = 0.16$, $P>0.05$). However, across all N treatments, mean numbers of alatae recorded after 7days (from 7.8 to 19.8 per plant) were consistently less than those recorded after 24 h (from 9.2 to 22 per plant) (Figure 5). From a total of 300 alatae released at the start in all five cages, 95% were found in the first assessment (i.e., after 24 h), of which 10% died (or were not found) in the second assessment (i.e., after 7 days).

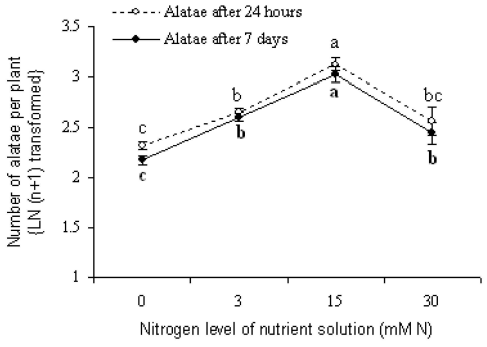


Fig 5. Mean ± s.e. numbers of adult alatae (*A. pisum*) recorded on host plants (*P. sativum*, var. Meteor) supplied with different nitrate-N concentrations in nutrient solutions (0, 3, 15 and 30 mM N); counts were made at 24h and 7d after releasing 60 adult alatae in a cage (n=5 cages); within a time, means with the same letter are not significantly different at 5% level (Duncan's multiple range test).

The number of alatae alighted on plants increased as N level increased, with the exception of the highest N level (30 mM N). Number of alatae settled on plants supplied with 30 mM N did not significantly differ from those plants supplied with 3 mM N, but these two N levels supported significantly less alatae than 15 mM N level ($P<0.05$). The least number of alatae was recorded on plants supplied with N-free solution (Figure 5).

Expressing number of alatae (after 7 days) per unit (cm^2) leaf area (i.e., population density of alatae) rather than "per plant", reversed the above comparisons among N treatments (compare Figure 5 with Figure 6). ANOVA results indicated that the number of alatae settled per unit area was highly significantly affected by levels of N supply ($F_{3, 16} = 30.21$, $P<0.001$). The numbers of alighted alatae per unit leaf area of the N-deficient plants were significantly greater than those of the N-fertilised plants. Density of alatae was least on plants supplied with the highest N level (30 mM). However, mean comparisons indicated that population density of alatae on the latter N treatment did not significantly differ from that of the 15 mM N treatment ($P>0.05$).

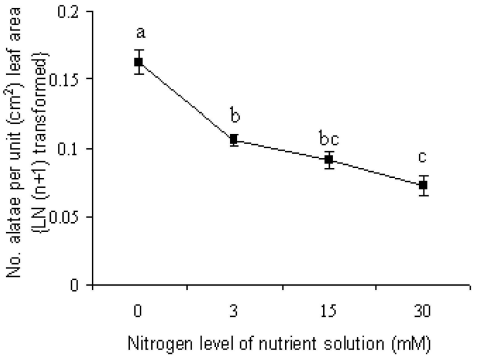


Fig 6. Density of *A. pisum* adult alatae, expressed as the mean number of adult alatae per unit leaf area (cm^2) recorded after 7 days access to host plants (*P. sativum*) supplied with different nitrogen concentrations in nutrient solution; initially 60 adult alatae had been released per replicate (cage) (n=5); bars represent standard errors; means followed by the same letter are not significantly different at 5% level (Duncan's multipl range test).

Alatae settled on each plant part were also recorded 24 h after release. The results indicated that the greatest numbers of alatae alighted on stipules and leaflets (80% of total number alighted), of which most (93%) were on the undersides, and that these parts contributed more than other parts to the differences observed among N treatments. One-way ANOVA within each plant part revealed that there was no significant difference ($P>0.05$) among N treatments with respect to number of alatae settled on either tendril, stem or shoot terminal (Figure 7). No alatae were recorded to have settled directly on visible buds.

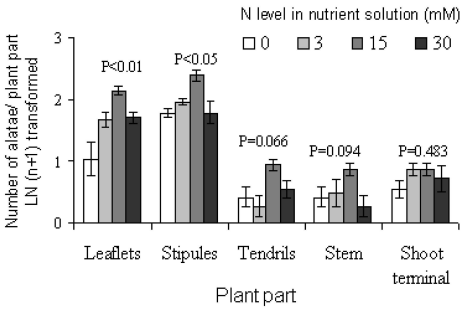
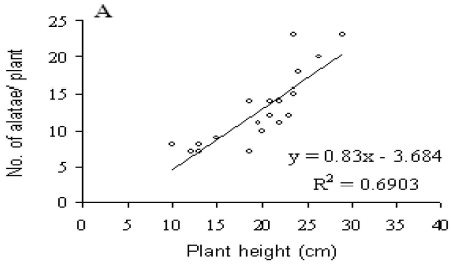


Fig 7. Mean ± s.e. number of adult alatae (*A. pisum*) recorded on different parts of host plants (*P. sativum*, var. Meteor) supplied with different nitrate-N concentrations in nutrient solutions (0, 3, 15 and 30 mM N); counts were made 24h after releasing 60 adult alatae in a cage (n=5 cages); P-values present results of ANOVA performed separately on each plant part for number of alatae present in different N treatments.

In addition, the effects of N-fertilisation mediated by plant size (shoot height or total leaf area) on settling preference of alatae were examined by regressing number of alatae (recorded after 7 days) on shoot height, and on total leaf area of the plants supplied with the respective N levels. Positive significant relationships were detected between number of alatae per plant and shoot height ($F_{1, 18} = 40.12$, $R^2 = 0.690$, $P<0.001$; Figure 8-A), or total leaf area ($F_{1,18} = 47.35$, $R^2 = 0.725$, $P<0.001$; Figure 8-B). However, numbers of alatae, expressed as counts per unit area, were negatively significantly correlated with the total leaf area ($F_{1,18} = 22.67$, $R^2 = 0.557$, $P<0.001$; Figure 8-C).



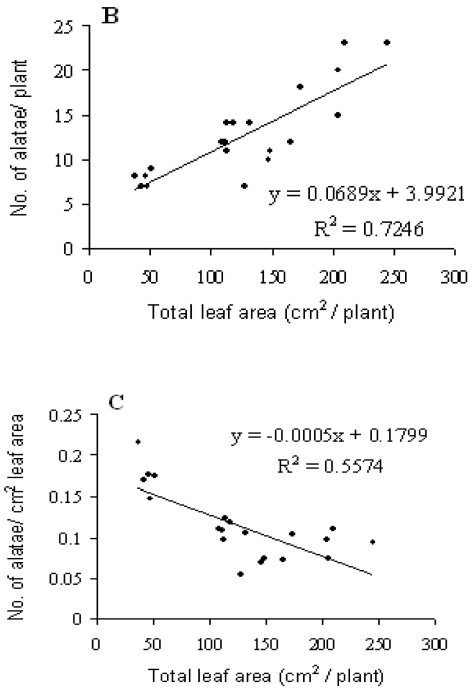


Fig 8. Scatterplots, regression lines and equations for numbers of adult alatae (*A. pisum*) settled per plant (*P. sativum*) on total leaf area (A) and plant height (B), 'C' as the same as 'B' but the vertical axis expressed as number per unit leaf area (n=20); within each of the five cages, 60 adult alatae had access for 7 days to the four plants each grown with one of the N levels (0, 3, 15 and 30 mM N, pooled data,).

Nymph deposition by adult alatae alighted

The number of offspring deposited for 7 days on each plant was highly significantly affected by the level of N supply ($F_{3, 16}$ = 25.35, P<0.001). The greatest number of nymphs was recorded on plants supplied with 15 mM N, and the least on those supplied with N-free solution (0 mM N); the difference between 15 mM N treatment and all other treatments was significant as indicated by Duncan's multiple range test (P<0.05). However, there were no significant differences between N-free solution and 3 mM N-solution, and between the latter and 30 mM N-solution in terms of number of nymphs per plant (Figure 9-A).

Nymph counts per plant were corrected for the number of alatae alighted (which had been recorded at the 7th day). The ANOVA results indicated that this parameter was significantly affected by N treatment ($F_{3, 16}$ = 6.93, P<0.01). The greatest numbers of nymphs were deposited by adult alatae that had alighted on plants supplied with 15 mM N solution, although not significantly different from 30 mM N. The trend and differences between N treatments in terms of nymphiposition, when corrected by number of alatae (Figure 9-B), differed slightly from those when uncorrected parameter was used (Figure 9-A). As an alternative to the above calculation (i.e., number of nymphs per alata per plant), analysis of covariance (ANCOVA) was conducted for nymph counts per plant in order to take into account of number of adult alatae (i.e., number of alatae per plant recorded at the 7th day was considered as a covariate). The results of this analysis indicated that the effects of N treatments were significant on the number of nymphs deposited by alatae (ANCOVA: $F_{3, 15}$ = 5.41, P<0.01). Using the sequential sum of squares from ANCOVA, it was found that 13.4% of the total variability in the number of nymphs per plant was explained by differences among the N treatments (supposedly mediated by feeding of alatae after alighting on plants), and 74.2% by differences in the number of alatae settled per plant.

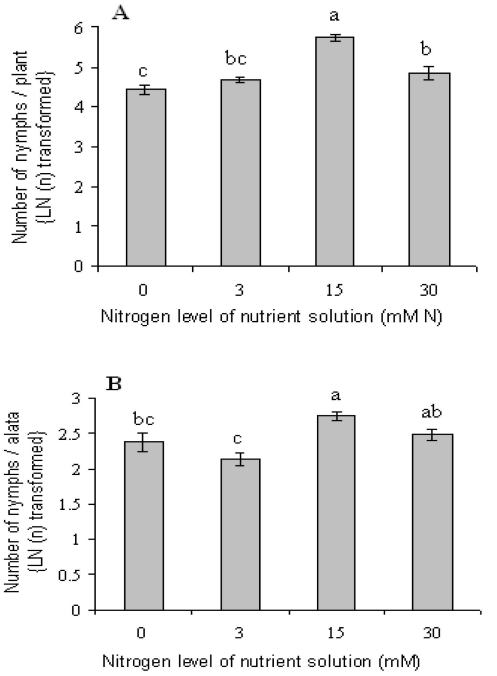


Fig 9. Mean numder of nymphs per plant (A) or per alata (B) recorded after 7 days access of alate *A. pisum* to the host plants (*P. sativum*) supplied with different nitrogen concentrations in nutrient solution; initially 60 adult alatae had been released per replicate (cage) (n=5); bars represent standard errors; means followed by the same letter are not significantly different at 5% level (Duncan's multipl range test).

Settling preference of alatae on leaf discs

Leaf discs were taken from plants, which had been grown with nutrient solutions differing in NO_3-N concentration, and were tested for preference of adult alatae. From total alatae released in six blocks, 83.3% participated in the preference test (i.e., alighted on leaf discs). The ANOVA results indicated that alatae settling was highly significantly affected by level of N in nutrient solutions ($F_{3, 20}$ = 17.14, P<0.001). Number of alatae alighted on leaf discs decreased as N supply increased, being greatest on leaf discs taken from plants supplied with N-free solution (2.92 ± 0.24, original data) and least on discs taken from plants supplied with 15 mM N (1.50 ± 0.13, original data) or 30 mM N (1.67 ± 0.11, original data). The latter two treatments were not significantly different from each other (P>0.05, Duncan's multiple range test). Spearman's rank-order correlation showed a strong, significant and negative association between settling preference of alatae and chlorophyll concentration of leaf (r_s = -1, n= 4, P<0.001), suggesting that alate aphids were attracted to chlorotic (yellow) leaf discs to a greater extent than to light-green and green or dark-green ones (Figure 10).

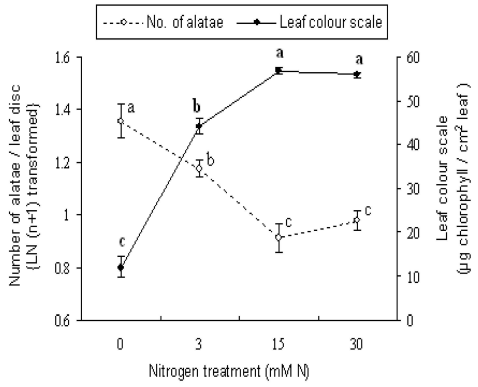


Fig 10. Number of adult alatae (*A. pisum*) (left axis), expressed as mean number per leaf disc (1 cm diameter) (n=6, each the average of 2 leaf discs), from host plants (*P. sativum*), recorded 24h after releasing of 20 adult alatae per replicate; leaf discs were taken from medium-aged leaves of plants supplied with different concentrations of nitrate-N (0, 3, 15 and 30 mM N); and the leaf colour scale of similar leaves (right axis), expressed as mean total chlorophyll content (µg per cm² leaf area) (n = 3). For a variable, means sharing the same letter are not significantly different at 5% level (Duncan's multiple range test); bars represent standard errors.

DISCUSSION

Peas (*P. sativum*) are considered to be less responsive to mineral nitrogen compared to most crops (Biddle *et al*., 1988). However, as the N levels applied in this experiment included the two extremes (0 mM and 30 mM N), the range was far greater than the tolerable range for plants in field conditions (normal conditions), and considerable changes in plant growth resulted. Examining the root of plants at the end of all experiments indicated that, as intended, no nodules were established, suggesting that growth was entirely reliant on nitrate-N source of nutrient solution. Many morphological and biochemical characteristics of plant are expected to alter due to varying N supply (Brouquisse *et al*., 2001; Lawlor *et al*., 2001), of which only a few were reported in this study. Many studies have demonstrated that the primary effect of N on crop production is via the formation of leaf area (e.g. Sinclair & Horie 1989; Van Keulen *et al*., 1989; Belanger *et al*., 1992). The present results indicated that shoot height and leaf area were significantly increased by nitrogen nutrition. Other growth components such as fresh or dry matter of foliage, although not reported here, were affected similarly. The relationships between these variables and the N level of nutrient solution were described far better by polynomial quadratic regression than by simple linear regression. Similar relationnships have been commonly found in the studies dealing with plant nutrition (Driessche & Webber 1975; Makowski *et al*., 1999; Lawlor *et al*., 2001; Ter Steege *et al*., 2001). The plausible explanation is that the amount of N taken up by roots and the efficiency with which is used in growth or yield (e.g., defined as yield/ N supplied) is not a constant, but changes with environmental factors and N supply. With no (or very small) N fertiliser application, uptake of N is small but efficiency large compared to large N applications (Lawlor *et al*., 1988; Lawlor *et al*., 2001; Ter Steege *et al*., 2001). Furthermore, in the present study, the growth of plants supplied with 30 mM N was adversely affected in comparison with those supplied with 15 mM N (Figure 2), in spite of that the former plants exhibited similar or slightly higher N soluble concentration than the latter (Figure 3). The results agree with the general inference of

Lawlor *et al*., (2001), stating that if N supply exceeds that required to maintain the potential growth rate, then no further dry matter is produced per unit additional N but more N may be accumulated in unproductive components; thus, N-use efficiency is small with ample N and decreases progressively and substantially as the N supply increases.

In herbaceous plants, particularly peas, aphids can feed on all aboveground parts. However, the abundance of alatae alighted was higher on leaves than on other parts (Figure 7), perhaps as the leaves constitute the greatest area of shoot (a leaf in conventional peas consists of one pair stipules, 1-3 pairs leaflets, and tendrils). Therefore, it was not surprising that total number of settled aphids per plant across N treatments was positively significantly correlated with total leaf area (Figure 8-B). Reports on the distribution of the pea aphid within plants note that the aphid was more often observed on leaves than on stems of broad bean (*Vicia faba*) (Salyk & Sullivan 1982). Physiological or ecological mechanisms have been mentioned as reasons for differences in distribution of an herbivore between plant parts. For instance, changes in feeding location can alter the access to nutrients thus influencing herbivore fecundity and development (Ralph 1976; Larson & Whitman 1991; Dixon 1998). Furthermore, herbivore behavioural responses could be important. Clegg & Barlow (1982) demonstrated that pea aphids feeding on plant stems were more responsive to alarm pheromone preceded by simulated vibration of plant substrate than aphids feeding on leaf undersides. In contrast, Legrand & Barbosa (2000) demonstrated that apterae of *A. pisum* did not discriminate between stems and leaves. It should be noted that all the aforementioned studies on within-plant distributions have been conducted on apterous *A. pisum*, which might differ in behaviour from alatiforms. The results indicated that fewer alatae settled on shoot terminals than leaves, and that abundance on the former did not differ significantly between different N treatments, as apposed to the abundance on the latter (Figure 7). This suggests that, regardless of the level of N supply, alate aphids always found shoot terminals a suitable (nutritious) site for feeding.

Moreover, a significant relationship was detected between numbers of alatae and height of plants (Figure 8-A). Such relationships have been reported in other studies. For instance, in a field study at a certain growth stage (29-37 day old plants) of 13 pea varieties, Cartier (1963) found that initial infestation by winged migrants (number of *A. pisum* alatae per row) was significantly positively correlated with plant height of varieties. However, it should be noted that in field condition, winged migrants may enter the crop every day and accumulate to reach a peak, then decline (such as the studies of Cartier 1963), but in controlled conditions (such as the present study) a fixed number of alatae is normally introduced to the plants once.

The positive effects of N supply or total soluble nitrogen (or free amino acids) of plants on growth and reproductive performance of aphids have been demonstrated in many studies (Maltais 1951; Auclair *et al*., 1957; Maltais & Auclair 1957; Van Emden 1966; Van Emden & Bashford 1969; Van Emden *et al*., 1969; Dixon 1970; Jansson & Smilowitz 1986; Febvay *et al*., 1988; Ponder *et al*., 2000). However, apparently no study has specifically focused on the relationship of nitrogen status of plant with the settling preference of winged aphids. In the present study, total soluble nitrogen was measured in a different series of plants from those used for the aphid settling preference experiment. However, as the growth conditions and procedure of plant culturing during the study were similar, the associations between mean values of the variables concerned can be sought.

Settling preference of alate aphids on plants was expressed as either aphid counts per plant (abundance) or aphid counts per unit leaf area (density). The trends observed in Figure 3 and Figure 5 suggest that the number of alatae settled per plant increased as concentration of soluble N in the leaves (or N supply) increased, although this association was not statistically significant (Spearman's rank-order correlation: $r_s = 0.4$, n= 4, $P>0.05$), mainly due to decreasing number of alatae settled on plants supplied with the highest N (30 mM N treatment) (Figure 5). Similar results in which the positive response of insects to N fertilisation did not correspond with the highest N supply in the

range of N treatments used, have been reported in other studies (cited by Bernays 1992). In a field study, Jansson & Smilowitz (1986) indicated that population growth rate of *M. persicae* was greatest on potatoes fertilised by a medium N application (140 Kg/ hectare), and decreased on plants fertilised with the higher (224 Kg/ hectare) or lower (56 or 84 Kg/ hectare) N applications.

If settling response of aphids is expressed as number of alatae per unit leaf area (Figure 6), then the association with soluble N (Figure 3) becomes significantly negative (Spearman's rank-order correlation: r_s= -1, n= 4, P<0.001), suggesting that the quality of food (at least in terms of concentration of total soluble N in leaf) might not have been the only factor influencing settling behaviour of the winged aphids. Perhaps quality of food in terms of the composition of nitrogenous compounds (e.g., essential amino acids compared to non-essential ones) is more important for aphids than the absolute concentration of total soluble nitrogen. Comparing the phloem sap of N-deficient with N-sufficient barely seedlings (*Hordeum vulgare* L.), Ponder *et al.*, (2000) demonstrated that the concentration of non-essential amino acids decreased due to decreasing N supply, but not the concentration of essential amino acids. This leads the ratio, essential/ non-essential amino acids to be higher in N-deficient plants than N-sufficient ones. Obviously, the nutritional quality of phloem sap or leaf will influence the alate aphids after settling on the plant and following probing of the substrate. The information about internal and physical properties of the substrate determines whether the aphid takes off and leaves the plant or whether it remains (Van Emden 1972; Dixon 1998).

Leaf chlorosis is a typical symptom of decreased N supply or decreased N content of the leaf (Taiz & Zeiger 1998), which is attributed to the decreased number and size of chloroplasts of the leaf (Kutik *et al.*, 1995; Lawlor *et al.*, 2001). The results indicated that leaf colour was affected by N level of nutrient solution, and was related to the total chlorophyll content of leaf (Figures 4 and 10). The associations among leaf colour, N supply or N content of leaf, and chlorophyll content have been demonstrated in many studies (e.g. Raese 1977; Pfeiffer & Burts 1984; Theobald *et al.*, 1998; Brouquisse *et al.*, 2001; Lawlor *et al.*, 2001). However, it should be noted that degradation of chloroplasts is only part of the many processes undertaken in leaf senescence (for a review see Brouquisse *et al.*, 2001). These processes are generally defined as proteolysis and nitrogen remobilisation. Leaf senescence is generally considered as the sequence of biochemical and physiological events comprising the final stage of development and leading to cell death (Nooden *et al.*, 1997). Senescence is genetically controlled, e.g., it will occur at a given time in the life of the leaf, even under optimal growth conditions: this is often called natural senescence (Smart 1994). However, senescence is also triggered by a number of unfavourable environmental conditions or by nutritional constraints (Brouquisse *et al.*, 2001) such as N deficiency in this study.

The results (Experiment 1) based on expressing the settling response of alatae as aphid counts per unit leaf area (i.e., density) indicated that significantly greater alatae settled on one cm^2 leaf of N-deficient plants than other N treatments (Figure 6), suggesting that the density of alate aphids alighted might be positively associated with yellowing of leaf colour, in spite of the lower nutritional quality of these plants (represented by lower soluble N content). The leaf area-based-expression of settling response, rather than plant based-, appears to be more relevant to ecological concepts (such as dispersal and host plant selection). In a similar manner in the large-scale studies of host plant selection by winged migrants (Kindlmann & Dixon 1994; Dixon 1998), the relative coverage of a plant species in the canopy is considered as a factor influencing the settling response. Furthermore, leaf area is often used as a base for expression of many physiological and physical characteristics of plants such as photosynthesis, chlorophyll and nutrients content, leaf thickness, etc, which might be more relevant to the density of insect populations (number per unit leaf area) than to abundance (number per plant).

The positive settling response of alatae to yellow was confirmed in the leaf disc experiment (Experiment 2), in which aphids were given a choice among equal-sized leaf discs taken from plants of the four N treatments (Figure 10). The N treatments resulted in a range of leaf colour from yellow

to green or dark green, which was quantified by total chlorophyll concentration of the leaf. Alatiforms of most aphid species are known to respond to visual cues, such as coloured surfaces of either host plants or inanimate objects (Folsom 1927; Moore 1935, 1937), and especially yellow (Kennedy *et al*., 1961; Cartier 1963; Dixon 1998). Dixon (1998) stated that "colour is a good indicator of the nutritive status of a plant, as both highly nutritious young and senescent foliage tend to be yellower than the nutritionally poorer mature leaves". However, although proteolysis in senescing organs of plants is a well-known process which may lead to increased quality of senescent leaves to aphid feeding compared to mature leaves, its enhancing effect on soluble nitrogen concentration of N-deficient plants, in the present study, might be not so great so as to exceed the positive effects of N-fertilisation. Furthermore, the literature shows contradictory results with respect to comparing the nutritional quality (in terms of soluble nitrogen or free amino acid concentration) of the foliages of the two plant strata, senescent vs. mature leaves. In the studies of Kennedy *et al*., (1950) and Kennedy (1958), it was suggested that the higher colonization of senescent (lower stadium) and growing (upper stadium) leaves, compared to mature green (middle stadium) leaves, in the same host plant by aphids (*M. persicae* and *Aphis fabae* Scopoli) might be due to the higher concentration of soluble organic nitrogen, although no analyses were done. Studies of Mittler (1954; 1958; 1958) on different growth stages of the willow tree (*Salix acutifolia* Willd.) indicated that the phloem sap had higher amino acid and total nitrogen contents when obtained from young or senescing organs than from mature leaves. In contrast, Jansson & Smilowitz (1986) on potatoes and Van Emden & Bashford (1969) on Brussels sprouts indicated that the concentration of soluble N or free amino acids in senescent organs was less than in mature or young leaves, although the authors emphasized the role of proteolysis in increasing the quality of senescent leaves for aphids. Van Emden & Bashford (1969) pointed out that high concentration of soluble nitrogen resulting from proteolysis might occur at a certain stage of senescence, which was not detected where tissue from several leaves was mixed before analysis. The results of the present study indicated that concentration of total soluble nitrogen of the leaves was signify-cantly lower in N-deficient plants than N-fertilised plants, and that the former had lower chlorophyll content than the latter. Therefore, the generalization of Dixon (1998) about the association between leaf colour and nutritional quality did not correspond with the present study, as nutritional quality of the yellow leaves (N-deficient plants), at least in terms of soluble nitrogen, was not better than light-green or green leaves (N-fertilised plants). The results also suggested that the better suitability of N-deficient plants than N-fertilised ones for settling of alate aphids (in terms of density) was less likely related to a nutritional factor, but more likely related to a visual factor, although the effect of other physical or biochemical factors (e.g., water content of leaves, secondary plant substances, and composition or concentration of leaf surface wax) on settling response of winged aphids cannot be ruled out.

The studies of feeding site-selection by aphids on host and non-host species or on susceptible and resistant varieties have indicated that the accumulation of aphids on certain species or variety is possibly due to a differential rate of departure, with aphids staying longer on their suitable host plants (Kennedy *et al*., 1959; Kennedy 1976). The rate of departure is determined by the aphids' response to external and internal features of the plant (Dixon 1998). Measuring minute by minute the behaviour of *A. fabae* on a susceptible and a resistant variety of *V. faba*, Müller (1958) found that both varieties were primarily infested by equal numbers of winged migrants and that the difference in preference arose exclusively from secondary choice through test feeding punctures, which did not take more than 20-40 seconds. The greater population on the susceptible variety resulted from the fact that alighted aphids remained a shorter time on the resistant plants. According to the results of Experiment 1 of the present study, although no such detailed observations were taken on the arrivals and departures of alate *A. pisum* on and from plants of the four N treatments, the highly significant correlation between numbers of nymphs deposited in 7 days per plant and the numbers of alatae settled after 24 h on all treatments ($F_{1, 18}$ = 95.58, R^2 = 0.842,

P<0.001; Figure 11) might suggest that alatae, after alighting on plants, stayed there to reproduce, and so did not leave immediately for reasons of preference. This was also confirmed by similarity of the number of alatae across N treatments at the two recording times, 24 h and 7 days after aphid release (Figure 5). Cartier (1963) in a field trial using pea varieties for preference test by *A. pisum* winged migrants demonstrated a significant correlation between number of colonies per row and number of winged migrants per row, based on which it was suggested that the aphids would not have taken off on the seedlings after they had settled on them. Powell & Hardie (2000) investigated host-selection behaviour by two winged phenotypes of *A. fabae*, gynoparae (adult autumn migrants which are monophagous) and alate virginoparae (summer winged aphids which are polyphagous) using close-up video technique, complemented with electrical penetration graph (EPG). They demonstrated that gynoparae discriminated beans from spindle within a 5-min period and many of them took flight from beans after a few (epidermal) stylet penetrations, whereas alate virginiparae behaved similarly on both plant species. Therefore, in the present study, perhaps the nutritional quality of N-deficient plants was not low enough to induce alatae to take off after landing on them, but was sufficiently low to decrease the reproduction of the alatae. Legrand & Barbosa (2000) reported that once an aphid (*A. pisum*) settled in one location on its host plant and began reproducing, it remains there from one to 7 days. Nevertheless, in the present study, as the aphids were not monitored during the 7 day-interval of the experiment, the occurrence of repeated landings or take-offs from a plant cannot be ruled out.

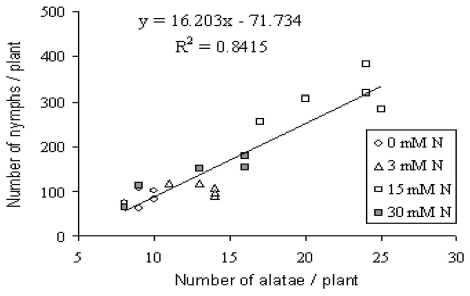


Fig 11 Regressing the total number of progeny (*A. pisum*) per plant (*P. sativum*) on the total number of alatae per plant (after 7 days) in different N treatments (0, 3, 15 and 30 mM N) (Experiment 1); Linear regression is presented; each point is representative of one replicate (plant), n=20.

The results of leaf disc experiment clearly showed that the yellow leaves of N-deficient plants (0 mM N) or the light-green leaves of moderately N-deficient plants (3 mM N) were preferred for settling by alate aphids after 24 h, compared to the green or dark-green leaves of the two other N treatments (15 and 30 mM N) (Figure 10). However, according to the results of the whole plant experiment, the alighted alatae, during 7-day test period, deposited fewer nymphs on plants of the two former treatments than on plants of the two latter ones (Figure 9). Therefore, with the assumption of no movements of aphids between plants during the experimental period, it appears that alate aphids did not have higher reproduction on their more-preferred plants. The decreased reproduction of aphids on the N-deficient or moderately N-deficient plants was considered to be due to the lowered total soluble nitrogen, compared to N-sufficient plants (i.e., 15 mM N treatment). The lower suitability of 30 mM N treatment compared to 15 mM N, in terms of preference for settling of alatae (Figure 6) or reproduction after settling (Figure 9), might be due to the higher accumulation of nitrate in leaf tissues. Manglitz *et al*., (1976) demonstrated that in seedlings of the resistant *Melilotus infesta* Guss., the nitrate ion plays the principal role in resistance to sweetclover weevil (*Sitona cylindricollis* Fahraeus) feeding. However, the deterrent or antifeedant effect of nitrate has not yet been reported for aphid feeding, nor was its concentration measured in plant materials of this study.

The results of preference test using leaf discs should be used with caution, because the physiological or biochemical changes in the leaves following excision were not taken into account. Comparing the effects of leaf excision on aphid growth performance and on chemical composition of the leaves in relation to the different nutrient (nitrogen and potassium) treatments of Brussels sprout, Van Emden & Bashford (1976) found considerable changes in total and composition of amino acids of the leaves, seven days after excision. For instance, they reported that concentration of total amino

acids in the leaf discs taken from 'high N low K' plants increased by 112% compared to concentration in the (attached) leaves from the similar treatment, but the concentration in the leaf disc from 'low N high K' plants reduced by 39% compared to the leaves of the same treatment. According to their previous study (Van Emden & Bashford 1971), the former plants were referred to 'susceptible' plants, and the latter to 'resistant' plants. They demonstrated that the use of leaf discs failed to reflect differences in 'nutritionally-based' plant resistance to *Brevicoryne brassicae* (L.) but not to *M. persicae*. In the present study, however, the growth of alate *A. pisum* was not the parameter being compared. Moreover, leaf discs were used immediately after cutting from plants and only for 24 h thereafter; therefore, the importance of physiological changes due to excision might be not so great as that in the studies of Van Emden & Bashford (1976). However, the effect of other non-assessed changes in the leaves after excision, such as water content or phloem pressure should have been considered, as feeding site selection or settling response of sap-sucking insects (including aphids) has been indicated to be influenced by physical status of the leaf (Kennedy 1958; Bernays 1992; Jauset *et al*., 1998). Therefore, comparisons between N treatments in terms of settling response of alate *A. pisum* (Figure 10) would be valid with the assumption that the magnitude and relative differences between N treatments in terms of physical and biochemical features were similar in (attached) leaves and leaf discs.

In conclusion, the laboratory-based results (free-choice experiments) providing evidence that the settling response of *A. pisum* alate adults on whole pea plant, and the subsequent reproduction of the alighted adults, are positively affected by the level of N supply or by the concentration of total soluble nitrogen of the leaves, with the exception of the highest N supply (30 mM N). However, the density of settled alatae (in terms of number per unit leaf area) increased as the level of N supply decreased, with the greatest density on N-deficient plants (0 mM N treatment) and lowest density on N-sufficient (15 mM N) or N-excess (30 mM N) plants. In addition, a free-choice experiment in which equal-sized leaf discs taken from the different N treatments were exposed to alate adults, indicated that the settling response of aphids was positively affected by leaf colour (yellowing), with the greatest number settled on yellow leaf disc (belonged to N-deficient plants) and smallest number settled on green or dark-green discs (belonged to N-sufficient and N-excess plants). Although the experiments of this study did not simulate precisely the field conditions in terms of the size of aphid population and the nutritional status of plants, they demonstrated the importance of N-fertilisation on settling response of winged migrant aphids, and might increase our understanding of host plant selection by winged aphids, particularly as there was no experimental evidence with regards to the effects of nitrogen status of a single plant species on settling response of this morph of aphids. Moreover, from the viewpoint of integrated aphid management, the results argued that although the positive effect of nitrogen fertilisation on growth and reproduction of aphids is a well-accepted phenomenon in the literature (Mattson 1982, 1993; Sandstrom & Moran 1999; Jansson & Ekbon 2002; Karley *et al*., 2002; Sudderth *et al*., 2005; Flynn *et al*., 2006), the strategy of "nutritionally-based" plant resistance focusing on "reducing nitrogen supply or content" cannot be a promising strategy for aphid management. The aphid population build-ups on these "so-called resistant" plants (i.e., N-limited plants) may be similar to the population on N-sufficient plants. Furthermore, density of aphids per unit leaf area or unit dry weight in N-limited plants will exceed that of N-sufficient plants, leading to reduced tolerance of the former to damage by aphid feeding. The results also emphasize that the plant breeding programmes for production of aphid-resistant varieties based on antibiosis will likely fail in practical integrated aphid management if the varieties are not examined for settling preference. It is possible that an antibiotic-resistant variety with yellower or pale green foliage (the foliage's colour due to either its morphological characteristics or nutritional imbalances) in field conditions may be infested by the same size of aphid population as a susceptible variety with green or dark-green foliage.

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