DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

Research and Development

CSG 15

Final Project Report

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Project title	Nutrient supply, growth and development of field vegetables.
DEFRA project code	**HH1408SFV**
Contractor organisation and location	Horticulture Research International, Wellesbourne, Warwick CV35 9EF
Total DEFRA project costs	£ 524,978
Project start date	01/04/99
Project end date	31/03/02

Executive summary (maximum 2 sides A4)

The UK horticulture and agriculture industries rely on large inputs of mineral nitrogen (N), phosphorus (P) and potassium (K) fertilisers to maintain product yield and quality. Recovery of applied fertilisers by field crops is inefficient (routinely < 50 % for N, < 10 % for P and < 40 % for K). Since unrecovered fertilisers are continually displaced through leaching and water / aerial erosion, adjacent terrestrial and marine environments are susceptible to nutrient enrichment (eutrophication). This can lead to habitat loss and a decline in biodiversity. Unrecovered fertilisers also represent an unnecessary cost to growers and there is also concern over possible health risks associated with the build up of residues (e.g. nitrates and certain trace minerals) in crop tissues. Thus, it makes environmental and financial sense to increase the nutrient-use efficiency of crop production by reducing fertiliser inputs whilst maintaining crop yields and quality.

The nutrient-use efficiency of a crop production system (yield per unit input of fertiliser) is determined by the environment in which the crop is grown and by the genetic characteristics of the crop plant (genotype). Nutrient-use efficiency can thus be increased by modifying the environment, or, by selecting and/or breeding varieties with enhanced nutrient-use efficiency. The overall aim of this project was to obtain (1) strategic information on the effects of nutritional supply on the growth and development of vegetable crops, and (2) preliminary strategic information on the effects of crop genotpye on nutrient-use efficiency. This aim was met through experimental work on a variety of crop and model plant species, grown in different nutritional environments.

We aimed to meet five specific Scientific Objectives:

1. To quantify the relationships between N, P and K supply, assimilation processes and morphological development.
2. To determine the spatial and temporal distributions of the main chemical forms of N, P, K and replacement elements, under reduced N, P and K supply.
3. To develop models of plant growth as a function of N, P and K supply, and tissue element concentrations.
4. To develop and extend the principles of standard growth analyses to establish new quantitative relationships between nutrient supply, whole-plant growth, plant development and crop yield.
5. To identify genetic loci impacting on P-uptake and utilisation (P-use efficiency), using model Brassicaceae systems *(Arabidopsis thaliana* and *Brassica oleracea*).

Within this project, we have delivered:

1. A detailed understanding of N, P, K supply, mineral distribution and growth in a model vegetable species (lettuce)

- Objective modelling techniques accounted for 99.0 and 99.1% of the variation in plant dry weight for control and N-limited plant growth respectively. For P and K experiments, fits accounted for more than 97 % of the variation in both treatments.
- Sub-linear relationships between nutrient concentration and relative growth rate (RGR) under restricted nutrient supply conditions conformed to a four-parameter dilution model.
- There were effects of all nutrient treatments on morphological and physiological components of growth.
- Detailed studies revealed that C assimilation decreased in N-limited plants, paralleled by decreases in stomatal conductance.
- Carbon assimilation was not directly limited by organic-N but by stomatal conductance.
- Reductions in stomatal conductance under N-limiting conditions were not linked to changes in stomatal distribution.
- Stomatal conductance is affected either directly back a lack of N, through an induced signal, or through compensatory feedback through increased intercellular CO_2. This remains an area of strategic uncertainty.

2. An understanding of the relationships between nutrient supply and the growth of whole-crops

- Almost all of the variation in crop growth as a function of 'physiological time' was explained using our chosen growth models.
- We used a four-parameter dilution model to predict shoot RGR as a function of shoot N and the model fitted the data well.
- The proportional effects of total-N and organic-N on shoot RGR were independent of plant age.
- The results from a whole-crop, grown to commercial maturity, are consistent with more detailed studies and thus supports the use of strategic studies to understand constraints to nutrient efficiency at the crop level.
- We can use these data to design assays to screen for nutrient-use efficiency traits in crop genetic resource collections.
- It is important to design assays to screen for nutrient-use efficiency traits that are appropriate to the environment in which the crop will be grown, and according to how nutrients will be supplied.

3. A preliminary characterisation of the genetics of nutrient-use efficiency in model Brassicaceae

- There is a significant genetic component to P-use efficiency(PUE) in the model Brassicaceae crops, *Arabidopsis thaliana* and *Brassica oleracea*.
- We have identified putative genetic loci impacting on PUE on Chromosomes I and IV in *Arabidopsis*, and on Linkage Groups 6, 7 and 9 in *Brassica oleracea*.
- We recommend that loci identified in *Arabidopsis* are fine mapped using emerging technologies.
- Genetic loci can be used to develop molecular markers for improving PUE in breeding programmes, or, can be used to screen existing germplasm for P-efficient varieties.

Within this project, we have published several primary papers in refereed journals (APPENDIX I). We have also ensured that knowledge and technology have been transferred from this work to fundamental and applied research groups, both within HRI and also in the international arena. Specific examples include (1) the delivery of data and ideas to applied projects to develop model-based fertiliser decision support systems, and (2) the testing of hypotheses on plant responses to mineral stresses (N, P and K) using genomic approaches. We have used data generated in this project to support applications for 'blue-skies', strategic and applied projects to the BBSRC, DEFRA and the Food Standards Agency.

By obtaining strategic information that may underpin a reduction in fertiliser-use, this project has met the primary DEFRA aim of sustainable development. It also meets the DEFRA specific policy objectives (i) to protect and improve the environment and enhance biodiversity, (ii) to promote a sustainable food supply chain, (iii) to promote sustainable, diverse, modern and adaptable farming and (iv) to promote sustainable management and prudent use of natural resources domestically and internationally.

Scientific report (maximum 20 sides A4)

1. AIMS & OBJECTIVES

The nutrient-use efficiency of a crop production system (yield per unit input of fertiliser) is determined by the environment in which the crop is grown and by the genetic characteristics of the crop plant (genotype). Nutrient-use efficiency can thus be increased by modifying the environment, or, by selecting and/or breeding varieties with enhanced nutrient-use efficiency. **The overall aim of this project was to obtain (1) strategic information on the effects of nutritional supply on the growth and development of vegetable crops, and (2) preliminary strategic information on the effects of crop genotpye on nutrient-use efficiency**. This aim was met through experimental work on a variety of crop and model plant species, grown in different nutritional environments.

Five specific Scientific Objectives were addressed:

1. To quantify the relationships between N, P and K supply, assimilation processes and morphological development.
2. To determine the spatial and temporal distributions of the main chemical forms of N, P, K and replacement elements, under reduced N, P and K supply.
3. To develop models of plant growth as a function of N, P and K supply, and tissue element concentrations.
4. To develop and extend the principles of standard growth analyses to establish new quantitative relationships between nutrient supply, whole-plant growth, plant development and crop yield.
5. To identify genetic loci impacting on P-uptake and utilisation (P-use efficiency), using model Brassicaceae systems (*Arabidopsis thaliana* and *Brassica oleracea*).

The Methods, Results and Discussion associated with Objectives 1-3 are presented in Section 2, whilst those associated with Objectives 4 and 5 are presented in Section 3 and Section 4 respectively. At the end of each section, we present a summary of the science and a summary of how we have transferred knowledge from the completion of these Objectives.

2. N, P AND K SUPPLY, DISTRIBUTION AND GROWTH OF LETTUCE (OBJECTIVES 1-3)

To meet Scientific Objectives 1-3, a series of experiments were conducted on crisphead lettuce plants, grown in a soil-free media (hydroponics). Plants grown in hydroponics are particularly effective at recovering nutrients from the external solution, even at low concentrations. Therefore, plants were grown under either nutrient-replete, or nutrient deficient (lacking one nutrient) conditions by withholding N, P or K during plant vegetative growth. During this period, plants were thus forced to rely on a restricted supply from their own internal nutrient reserves. Measurements of growth, and components of growth, were compared with a control treatment in which all nutrients were supplied continuously throughout. Work associated with these Scientific Objectives has been published (Broadley et al., 2000; Broadley et al., 2001a, 2001b; Broadley et al., 2002a); the generic methods are described below.

2.1 Methods

2.1.1 Plant material and growth conditions

Experiments were carried out in a glasshouse compartment set to 25/15 °C day/night. Natural daylight was supplemented using high-pressure 400 W Na-vapour lamps to provide a 15 h day^{-1} light period. Lettuce (*Lactuca sativa* L. cv. Saladin R100) plants, supplied as pelleted seed (Elsoms Seeds Ltd, Spalding, Lincs., UK), were raised in perlite and irrigated with a half-strength nutrient solution (Table 1). At the 4-5 leaf stage, plants were transferred into hydroponic units containing a full strength nutrient solution at full strength. All plants then remained in the nutrient solution until a period of time after sowing (between 6 and 8 weeks, according to the time of year) when a second, nutrient-limiting, treatment was imposed by switching half of the hydroponic units to a solution lacking one nutrient. The nutrient solutions were monitored at least twice weekly and adjusted for nutrient concentration by replacing solutions when necessary (weekly). No significant nutrient depletion was observed in any of the control nutrient solutions. Solutions in each tank were aerated continuously.

2.1.2 Experimental design

Each hydroponic unit was constructed from a 33 litre polypropylene tank with a rest-on lid, connected in parallel to each of the others within a treatment by a series of input and output pipes. A pump was used to circulate the nutrient solution around this system at a rate of 50 ml s^{-1}. Each plant was suspended through a separate hole in the tray lids so that plant roots were fully submerged in the nutrient solution. Each plant was secured by a foam-rubber plug to hold the stem in an upright position while allowing it to expand freely during growth. This arrangement allowed destructive sampling, without damage to other plants, throughout the experiment. There were eight separate hydroponic units (tanks) arranged in a 4 x 2 array with treatments systematically allocated to pairs of tanks in a 2 x 2 design. At each sampling date, one plant was removed at random from each tank. All morphological and physiological measurements were made on this plant (i.e. four plants per treatment). Analyses of variance were performed to assess the effects of treatment, and also effects of sampling date within treatment, assuming a split-plot type design: treatments applied to main plots (tanks), whilst sampling date applied to sub-plots (plants within tanks). No estimate of variation was made across the experimental

area although the treatments were arranged to minimise any variation. Statistical analyses were performed using Genstat (Fifth Edition, Release 4.2, VSN International, Oxford, UK).

2.1.3 Plant growth rates and components

Absolute (AGR) and relative (RGR) growth rates were estimated from the data using the RSCHNUTE procedure in Genstat (Burns et al., 1997; Keen, 2000). However, since plant growth is the integral of numerous physiological and morphological processes, components of RGR were also estimated. The morphological component of growth is defined in terms of the leaf area ratio (LAR) between leaf area and plant weight. In turn, the LAR represents the product of the specific leaf area (SLA), leaf area per unit weight of leaf, and the leaf weight to plant weight ratio, or leaf weight ratio (LWR). The physiological component of growth is the net assimilation rate (NAR), the amount of C assimilated per unit time per unit area of leaf. The NAR represents the difference between photosynthesis and respiration (van der Werf, 1996). To determine these components of growth, detailed morphological and physiological measurements were taken, and these are described in 3.1.4 and 3.1.5.

2.1.4 Morphological measurements

Measurements of the weights and areas were made on each leaf of a size greater than about 625 mm^2. Leaf areas were obtained using a flatbed scanner and HP Deskscan II software (ScanJet 4p, Hewlett Packard Co., USA), with images measured using Sigma Scan 3.02 software (Jandel Scientific Software, Germany). From these measurements, plant SLA and LWR were calculated on a dry weight basis. Leaf areas were obtained from leaf images scanned either directly, or indirectly after being photocopied, into a PC using a flatbed scanner (ScanJet 4p, Hewlett Packard Co., USA) and HP Deskscan (Version II, Hewlett Packard Co., USA) software. Cell numbers and areas were obtained from impressions taken from the adaxial surface of the leaves sampled during the course of each experiment. A thin layer of 'Copydex' adhesive (Henkel, Cheshire, UK) was applied to c. 0.5 cm^2 of each leaf taken for analysis and allowed to dry. Microscope slides were prepared by covering with a thin layer of 'Copydex' and allowing to dry. This leaf area, covered with dried 'Copydex', was carefully pressed onto the dried 'Copydex' on the slide and peeled away. This left an impression of the epidermal cells on the slide. Cell impressions taken this way were scanned onto the computer using a video camera (DXC-151P, SONY, Japan) attached to a light microscope, and the image grabbed using WinTV (Version 1.6, Hauppauge Computer Works, Inc., USA) software. Leaf and cell areas, and leaf numbers were calculated following image manipulations using Paint Shop Pro (Version 4.12, JASC Inc., USA) and Sigma Scan (Version 3.02, Jandel Scientific Software, Germany). From these data, stomatal frequency (number of stomata per unit area) and stomatal index (SI: the ratio of stomata to epidermal cells per unit area) were calculated (Salisbury, 1927).

2.1.5 Gas exchange measurements

Rates of C assimilation and the conductance of stomata to water vapour were measured using a portable infra-red gas analyser open system fitted with a 625 mm^2 broad-leaf stirred cuvette (LCA-4, Analytical Development Co., Herts., UK). Measurements were made *in-situ* on each plant at intervals starting from the day on which treatments were imposed. Measurements were made on every leaf with sufficient area to fill the leaf cuvette, under 1000 µmol m^{-2} s^{-1} of photosynthetically active photon flux rate (PPFR) using a light-unit attached to the leaf cuvette. The air flowing through the cuvette was pumped from the outside of the glasshouse. To ensure rapid but accurate measurements of the large numbers of leaves on all sampled plants, a protocol that was designed to take "snapshot" readings was followed (Parsons et al., 1997). Readings were taken every 30 s over a period 90 to 180 s after clamping the leaf in the cuvette and the individual leaf mean values calculated. The whole-plant mean rates of C assimilation and stomatal conductance were estimated from mean individual leaf values, weighted by leaf area. In selected experiments, gas exchange was measured under more detailed conditions and the response of C assimilation and stomatal conductance to light was measured on two or three mature leaves per plant (including the last fully-expanded leaf) under nutrient replete and nutrient deficient conditions. PPFR at the leaf surface was varied between 29 and 1253 µmol photon m^{-2} s^{-1}, using a series of neutral density filters fitted to the light-unit attached to the leaf cuvette (Parsons et al., 1997). Irradiances at the leaf surface were determined by positioning the LCA-4 PAR sensor at the centre of the cuvette and measuring the radiation transmitted through each filter. Gas exchange rates were recorded every 30 s for at least three minutes once steady-state CO_2 exchange was attained. At each PPFR, the arithmetic mean of between four and seven readings was calculated. A three-parameter model was fitted to the light response curves for each leaf (Equation 1).

$$A = \frac{A_{\max} . (I - I_{\min})}{K_m + (I - I_{\min})} \qquad \text{Equation 1}$$

In equation 1, A is the C assimilation rate (µmol C m^{-2} s^{-1}), I is the PPFR (µmol photons m^{-2} s^{-1}), $A_{\max}$ is the maximum A (as I tends to infinity), K_m is the value of I when A is ½ $A_{\max}$ and $I_{\min}$ is the value of I at which A reaches 0 (the light compensation point). In order to estimate the time needed to reach maximum g_s, stomata closure was induced in one plant from each treatment by maintaining them in the dark for 1 h before measurements (Parsons et al., 1997).

2.1.6 Plant mineral element concentrations

Total N was measured using $^{15}NH_4{}^{15}NO_3$ (supplied to the plants at a ^{15}N atom percent enrichment of 2.75% until two treatments were imposed) to faciliate measurements on small individual leaves of plants. ^{15}N was supplied to all plants from sowing until 47 days after sowing (DAS). In these plants, total-N analyses were performed by mass-spectrometry on sub-samples of 0.5–1.5 mg dry plant tissue (Mylnefield Research Laboratories, Dundee, UK). Nitrate-N was measured using the extraction procedure outlined in Hunt and Seymour (1985). Briefly, a maximum of 0.2 g sub-sample of homogeneous, milled, dry leaf tissue was shaken for 30 min in a flask containing 50 ml deionised H_2O and 200-300 mg of activated charcoal. The solution was filtered through Whatman No. 1 filter paper, with the initial 2 ml of filtrate discarded. The filtrate was analysed for nitrate-N using a continuous flow colormetric method on a flow-injection analyser (FIASTAR 5012, FOSS Tecator, Sweden). The amount of deionised water used was adjusted for small samples. Organic-N represents the difference between total-N and nitrate-N. For P analyses, plants were dried to constant weight for between 1 and 10 min in a 650 W microwave to minimise the hydrolysis of structural P molecules (Bollons and Barraclough, 1997). Milled plant samples were divided and analysed separately for storage/metabolic P and total P concentrations. A procedure which combines inorganic and organic forms of storage/metabolic P was selected, as both forms of P function as a "storage/metabolic" pool during luxury accumulation of P. The storage/metabolic P pool was extracted from dry plant tissue using either 10 ml or 25 ml of acetic acid 2% (v/v) to extract P from samples less than or greater than 0.1 g dry weight, respectively. Samples were shaken in glass jars for 30 min and filtered through Whatman No. 5 paper; the initial filtrate was discarded. Total plant P measurements were obtained using the micro Kjeldahl method with samples digested for 1 h at 330 °C following addition of 1 ml of H_2O_2 and 2 ml of a H_2SO_4/Se catalyst. Phosphorus concentrations on extracted or digested P material were determined using inductively-coupled plasma atomic emission spectrophotometry (JY24, Jobin-Yvon ISA, France). Structural P represents the difference between total P and storage/metabolic P. To assess the effects of microwave versus conventional oven drying, additional plants were sampled at seven sampling times, and subjected to a conventional drying procedure for 24 h in a fan-assisted oven set to 80°C. Phosphorus analyses were carried out as before. In the K experiment, plant material was digested using a micro-Kjeldahl digest. Dried samples of between 0.11-0.13 g were used, 2 ml of H_2SO_4 / Se mixture catalyst and approximately 1 ml of H_2O_2 were added and the sample placed on the digestion block (Gerhardt KBL 40s, Brackley, Northants., England) at 330 °C for 45 minutes. For smaller samples the amount of H_2SO_4/Se added was adjusted. Samples were coolled, more H_2O_2 added, and the samples digested for a further hour. The samples were then diluted 1:25 with deionised water and mixed thoroughly. A further 1:10 dilution was made with a 3 mM Li solution. The samples were then analysed for K and Na using a flame photometer (FLM3 Flamephotometer, Radiometer, Copenhagen, Denmark).

Figure 1. Hydroponic system used for lettuce N, P and K experiments; this system allows root samples to be analysed seperately.

2.2 Results & Discussion

The removal of N supply led to a greater, and a more rapid, reduction in growth than observed for P and K. Thus, to determine the precise impacts of nutrient removal on components of growth, we focus here on data derived for N. Effects of P and K on plant growth have been published during the course of this project (Broadley et al., 2001b, 2002a).

2.2.1 Relationships between N and growth in lettuce.

Measured dry weights increased in both treatments throughout the experiment, although increases were slower in plants that became N-limited following removal of external N. In both treatments, the Richards' growth function was automatically selected by the RSCHNUTE procedure (Fig. 1). Whole-plant N concentrations decreased more rapidly in the plants deprived of N than in the control plants, with significant differences between treatments from day 49 onwards (Fig. 2). Total amounts of N (g plant^{-1}) remained constant in plants that were N-limited, but continued to accumulate in control plants (Fig 3a). The corresponding relationships between RGR and the concentration of N in plants are shown in Fig. 3b. A four parameter model, which assumes that the decline in shoot RGR after an interruption in N supply is a linear function of the reciprocal of the total-N concentration of the shoot, and that the redistribution of N from shoots to roots is minimal, was fitted to estimates of RGR as a function of plant N concentration, for N-limited plants (Equation 2).

$$RGR_{shoot} = \frac{1}{W_s} \cdot \frac{dW_s}{dt} = \mu_s \left(1 - \frac{W_{s0} \cdot [N_T]_{s0}}{W_{sf} \cdot [N_T]_s} \right) \qquad \text{Equation 2}$$

In Equation 2, t is the time from when the external N supply was withheld, W_s is the weight of the shoot at time t, W_{s0} is the value of W_s at $t = 0$, W_{sf} is the final asymptotic value of W_s when growth stops due to acute N deficiency (where $W_{sf} > W_{s0}$), $[N_T]_s$ is the concentration of total N in the dry matter of the shoot at time t and $[N_T]_{s0}$ is the value of $[N_T]_s$ at $t = 0$, and μ_s is a logistic growth constant. This model fits the data well ($r^2 = 0.97$; $P < 0.001$).

Fig. 2

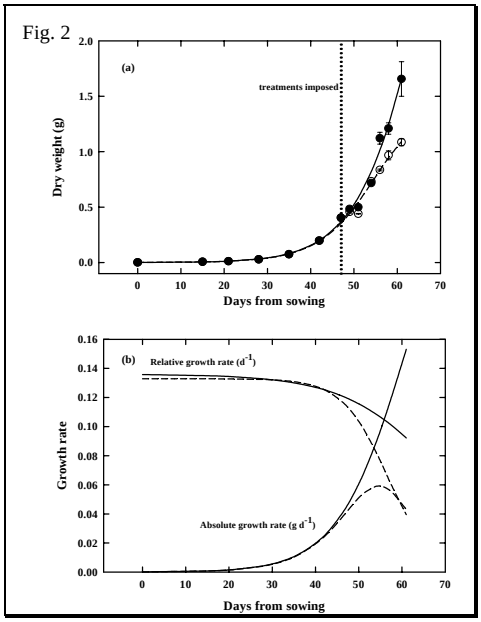


Fig. 2 (a) D. wt of *Lactuca sativa*. Filled circles and solid line, control plants. Open circles and the dashed line, N-limited plants, whose external N supply was removed on day 47. Mean of four replicates ± 1 S.E.M. Curves fitted using RSCHNUTE procedure in Genstat. **(b)** Estimated absolute and relative growth rates, obtained from the curve fitting presented in (a), expressed as a function of time.

Fig. 3

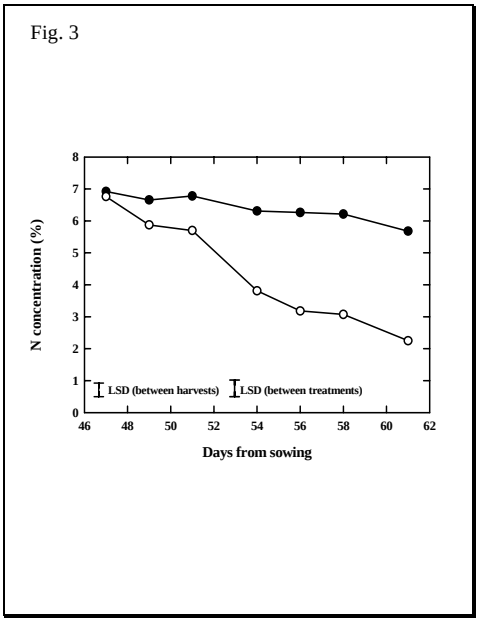


Fig. 3 Nitrogen concentration of *Lactuca sativa*. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47. Mean of four replicates. Least significant differences ($P = 0.05$) are between harvests within a treatment, and between treatments at each harvest.

Fig. 4

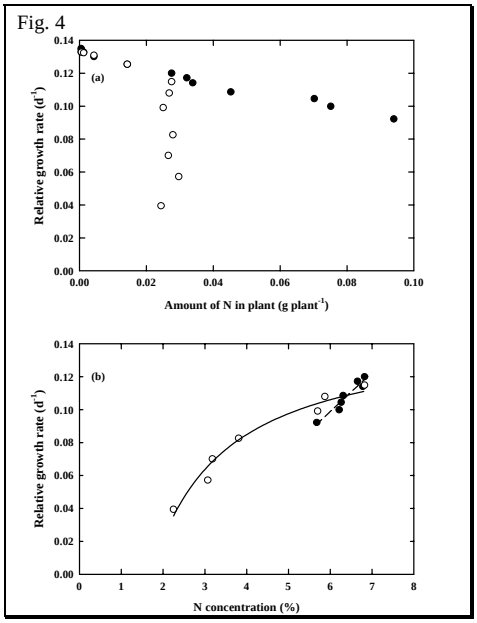


Fig. 4 Relative growth rates of *Lactuca sativa* vs **(a)** amount, **(b)** concentration of N. Filled circles, control plants. Open circles, N-limited plants, whose external N supply was removed on day 47. Control plants: regression line is linear least square fit, $y = 0.024x - 0.044$ ($r^2 = 0.91$; $P < 0.001$). N-limited plants, Equation 2 was fitted, $y = 0.1484*(1-((6.822*0.404)/1.6x))$ ($r^2 = 0.97$; $P < 0.001$). Nitrogen data is mean of four replicates.

2.2.2 Relationships between N and components of growth in lettuce.

The precise effects of N deprivation on morphological components of growth are presented (Fig. 5). Leaf weight ratio (LWR) increased over time in control plants and decreased in N-limited plants, creating significant differences between treatments from day 54 (Fig. 5a). Specific leaf area did not change significantly over time either in plants receiving N or in plants deprived of N, nor was it significantly different between these treatments (Fig. 5b). Shoot to root biomass allocation increased over time in control plants but did not change significantly in the N-limited plants (Fig 5c). There were no relationships between plant N concentration and SLA or LWR (data not shown).

N deprivation also affected physiological components of RGR (Fig. 6). Whole-plant C assimilation rates remained steady from day 47 in control plants, decreasing only at the final sampling date. However, significant decreases in N-limited plants occurred 7 d after withholding N, resulting in between-treatment differences in the rate of C assimilation from day 54 (Fig. 6a). Similar differences in stomatal conductance were observed between treatments, with N-limited plants having lower stomatal conductances than control plants (Fig. 6b). In N-limited plants, there was a positive linear relationship between the concentration of N and the mean whole-plant assimilation rate for C ($r^2 = 0.8$; $P < 0.01$). It is not possible to observe any relationship between N concentration and C assimilation in control plants (Fig. 7).

Table 1 Nutrient solutions used in experiments

	Concentration in Nutrient Solution (mM)+			
	Full Nutrient Solution	-N	-P	-K
$Ca(NO_3)_2.4H_2O$	2	-	2	2
NH_4NO_3	2	-	2	2
KH_2PO_4	0.25	0.25	-	-
KOH	0.5	0.5	0.5	-
$MgSO_4.7H_2O$	0.75	0.75	0.75	0.75
$CaCl_2.2H_2O$	0.025	0.025	0.025	0.025
FeNaEDTA	0.1	0.1	0.1	0.1
H_3BO_3	0.03	0.03	0.03	0.03
$MnSO_4.4H_2O$	0.01	0.01	0.01	0.01
$ZnSO_4.7H_2O$	0.001	0.001	0.001	0.001
$CuSO_4.5H_2O$	0.003	0.003	0.003	0.003
$Na_2MoO_4.2H_2O$	0.0005	0.0005	0.0005	0.0005
$Ca(H_2PO_4)_2$	-	-	-	0.27
$CaSO_4.H_2O$	-	4	-	-
K_2SO_4	-	-	0.125	-

+ adjusted to pH 5.5-6.5 using H_2SO_4/$Ca(OH)_2$ as appropriate

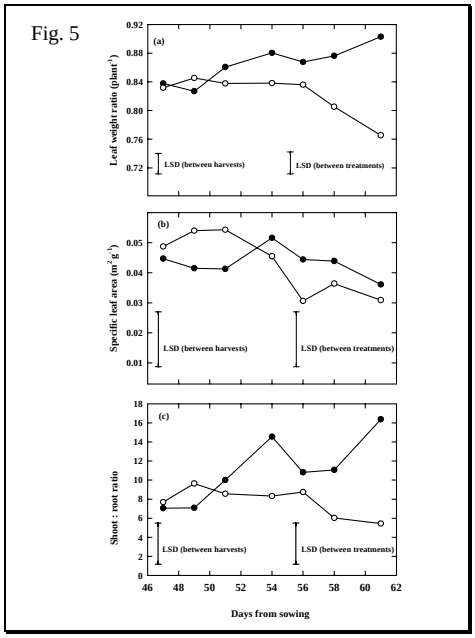


Fig. 5 Components of growth: **(a)** leaf weight ratio **(b)** specific leaf area **(c)** shoot : root ratio, of *Lactuca sativa*. Closed circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47. Each point is the mean of four replicates.

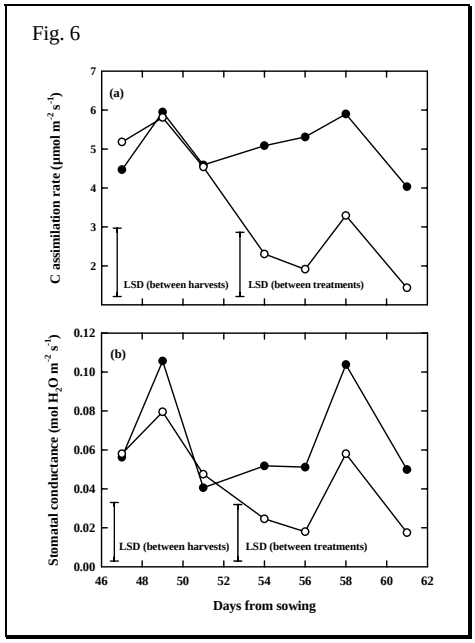


Fig. 6 Carbon assimilation rate **(a)** and stomatal conductance **(b)** of *Lactuca sativa*. Closed circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47. Each point is the mean of four replicates.

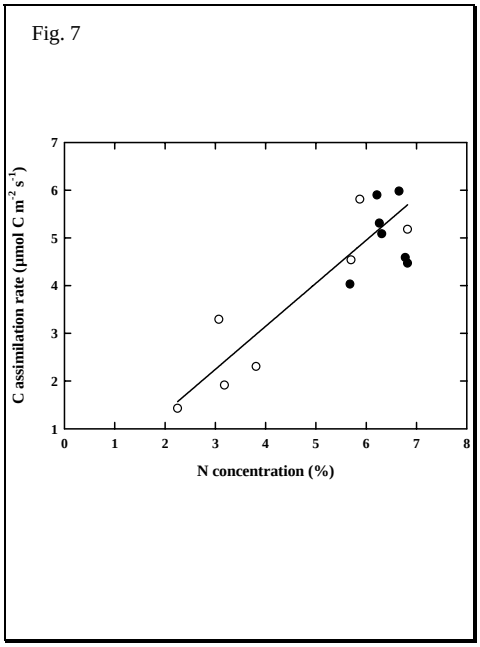


Fig. 7 Carbon assimilation rate as a function of plant N concentration. Closed circles, control plants. Open circles, N-limited plants, whose external N supply was removed at day 47. Regression line is the linear least square fit, $y = 0.861x - 0.323$ ($r^2 = 0.80$; $p < 0.01$).

2.2.3 Relationships between N and detailed physiological components of growth in lettuce.

Instantaneous estimates of A were related sub-linearly to g_s, although the distribution of data differed in N-limited plants; as N-deficiency increased, N-limited plants had lower stomatal conductance values than N-sufficient plants (see Broadley et al., 2000 for details). The responses of different leaves to PPFR are presented in Fig. 8 (steady-state gas-exchange). Equation 1 was fitted all of the 16 curves; all 16 fits had a r^2 of at least 98.4 %. No photoinhibition was observed at high irradiance. Control plants had a higher A_{max} than N-limited plants (t = 3.42, 14 d.f., $P = 0.004$). A_{max} of control and N-limited plants was 13.09 ± 0.72 (n = 9) and 9.06 ± 0.97 (n = 7) respectively (mean ± SEM). There were no significant differences in K_m or I_{min} between treatments. K_m was 242.2 ± 10.45 and 218.6 ± 18.33 and I_{min} was 35.05 ± 1.53 and 36.61 ± 1.62 for control and N-limited plants respectively. Stomatal conductance reached steady-state (stomata were at maximum aperture) in similar times for both treatments, c. 50 min in plants darkened previously for 1 h. In the leaves presented in Fig. 8, there was no effect of N on the relationship between g_s and A at low levels of g_s (Fig. 9). However, leaves of N-limited plants had lower values of g_s than control leaves. Thus, although estimated A_{max} values and leaf organic-N concentration were positively related in leaves of a similar developmental state (Fig. 10), when A as a function of organic-N is presented at three selected values of g_s (0.05, 0.1 and 0.15 mol H_2O m^{-2} s^{-1}), there is no treatment effect on A (Fig. 11). Although leaves from control plants contained more organic-N than in N-limited plants (mean organic-N levels for control and N-limited plants was 5.44 % and 2.65 % (0.59 LSD at $P = 0.05$) respectively), there was no organic-N limitation of A.

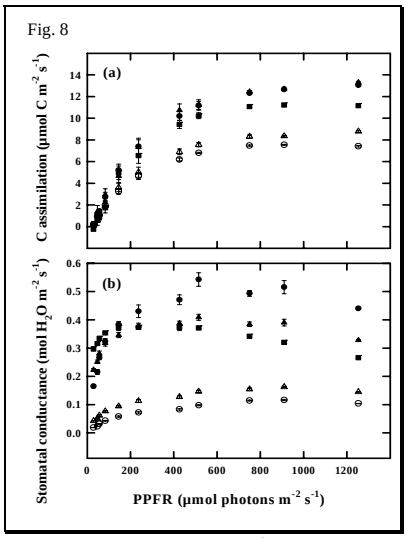


Fig. 8 Photosynthetic responses of *Lactuca sativa* to PPFR. **(a)** C assimilation **(b)** stomatal conductance. Filled symbols, control plants. Open symbols, N-limited plants whose supply of N was removed on day 47. In control plants, circles, triangles and squares are leaves 8, 10 and 11 (youngest fully-expanded leaf) respectively. In N-limited plants, circles and triangles are leaves 9 and 10 (youngest fully-expanded leaf). The curves were obtained on day 56. Each point is the mean of four-to-seven IRGA readings (± SEM).

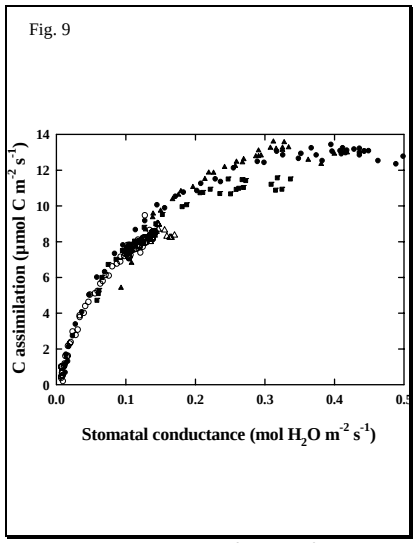


Fig. 9 C assimilation as a function of stomatal conductance in *Lactuca sativa* leaves at PPFR > 900 µmol photons m^{-2} s^{-1}. Filled symbols, control plants. Open symbols, N-limited plants whose supply of N was removed on day 47. In control plants circles, triangles and squares are leaves 8, 10 and 11 (youngest fully-expanded leaf) respectively. In N-limited plants, circles and triangles are leaves 9 and 10 (youngest fully-expanded leaf). The curves were obtained on day 56. Each point is an individual IRGA reading.

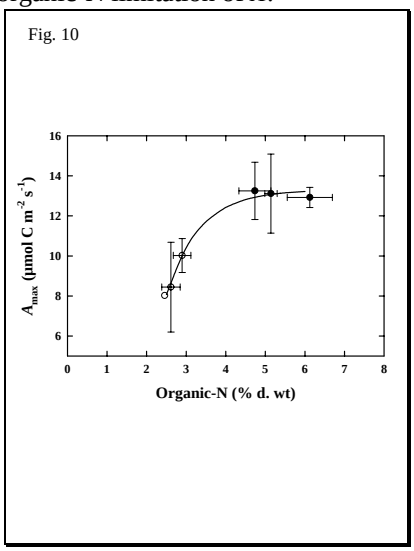


Fig. 10 Maximum photosynthesis rate (A_{max}) as a function of organic-N concentration in *Lactuca sativa*. Two or three leaves were sampled per plant (including the most recent fully-expanded leaf) on days 56 and 58. Filled symbols, control plants. Open symbols, N-limited plants whose supply of N was removed on day 47. Error bars are SEMs. A function was fit to means ($y = 13.3 - 102.5 * 0.3042^x$; $r^2 = 0.98$; $P = 0.001$).

2.2.4 Relationships between N and detailed morphological components of growth in lettuce.

Total leaf area increased faster in control than in N-limited plants (Fig 12a). There was no difference in total stomata between adaxial and abaxial leaf surfaces (Fig. 12b); linear regression between adaxial and abaxial stomatal numbers, constrained through the origin, was highly significant ($y = 0.983x$; $P < 0.001$; $r^2 = 0.90$). Therefore, all subsequent analyses were restricted to adaxial epidermal layers. Although total leaf area increased during control plant development, stomatal frequency and stomatal index (SI) remained constant. Stomatal frequency is variable and influenced by environmental effects and a more stable method of describing the epidermal-stomatal complex is SI. There were no differences in stomatal frequency or SI between control and N-limited plants (Fig. 12c,d). Thus, N did not affect the epidermal-stomatal complex. Although observations were made on leaves formed prior to onset of N starvation, these leaves make up the bulk of a plants photosynthetic apparatus and will dominate observed growth responses.

Fig. 11

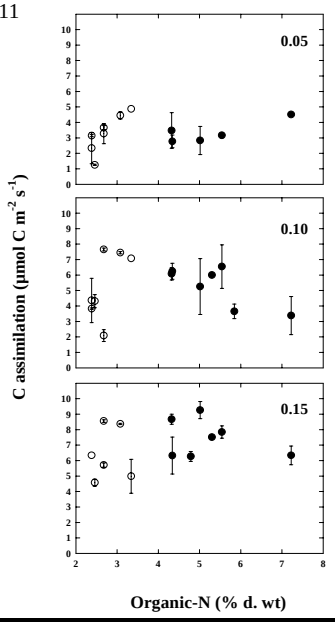


Fig. 11 C assimilation as a function of organic-N in *Lactuca sativa*. Data are IRGA measurements (mean ± SEM) at three rates of g_s (0.05, 0.1 and 0.15 mol H_2O m^{-2} s^{-1}). Two or three mature leaves were sampled per plant (including the most recent fully-expanded leaf) on days 56 and 58. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

Fig. 12

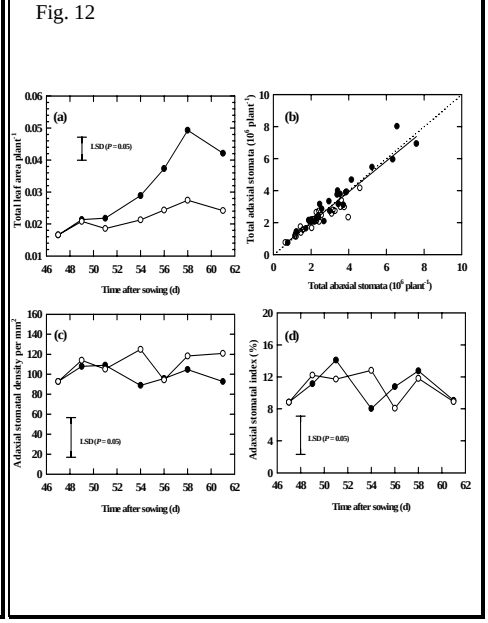


Fig. 12 Leaf morphological characteristics in *Lactuca sativa*. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47. (a) Total leaf area per plant (b) total number of stomata on adaxial and abaxial leaf surfaces (c) adaxial stomatal density (d) adaxial stomatal index.

Fig. 13

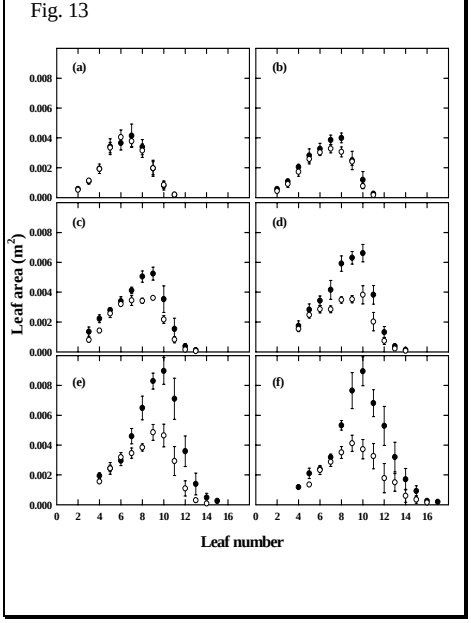


Fig. 13 Mean leaf area (± SEM; $n = 4$) of *Lactuca sativa* after (a) 49 (b) 51 (c) 54 (d) 56 (e) 58 and (f) 61 d growth. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

The areas of individual leaves are presented following imposition of treatments (Fig. 13). The maximum expanded leaf at the onset of treatments was leaf 7. Leaves above this insertion level continued to expand in control plants, and attained larger final areas than corresponding leaves in N-limited plants. To determine what morphological character underpinned these changes in leaf area, epidermal cell areas and the total number of epidermal cells per leaf were determined. Nitrogen-limited leaves had smaller cell areas than leaves from control plants in older and intermediate leaves (Fig. 14). There were no treatment differences in cell areas from leaves which expanded following the imposition of treatments (leaf 12 and above). The opposite response occurred in total numbers of epidermal cells per leaf. Leaves which expanded prior to the imposition of treatments (leaf 11 and below), showed no further changes in number of epidermal cells per leaf either during subsequent plant development, irrespective of N treatment (Fig. 15). However, leaves that expanded after this time had significantly fewer epidermal cells per leaf.

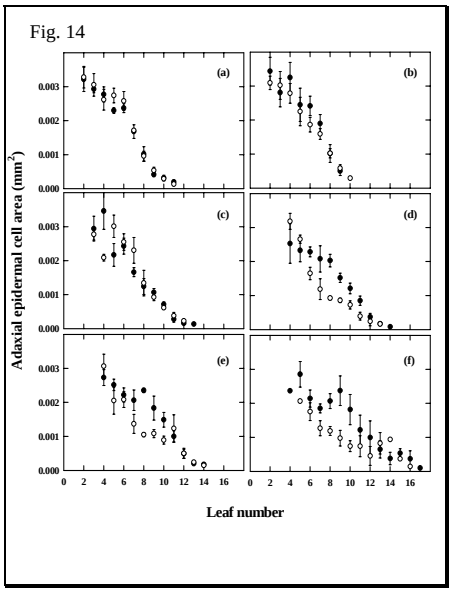


Fig. 14 Mean adaxial epidermal cell area (± SEM; n = 4) in *Lactuca sativa* after (a) 49 (b) 51 (c) 54 (d) 56 (e) 58 and (f) 61 d growth. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

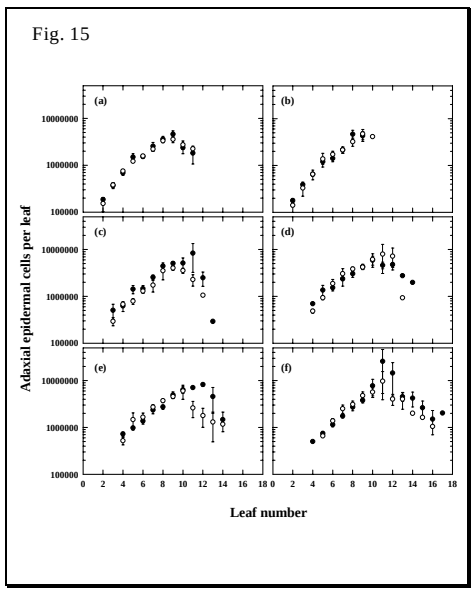


Fig. 15 Mean number of adaxial cells per leaf (± SEM; n = 4) in *Lactuca sativa* after (a) 49 (b) 51 (c) 54 (d) 56 (e) 58 and (f) 61 d growth. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

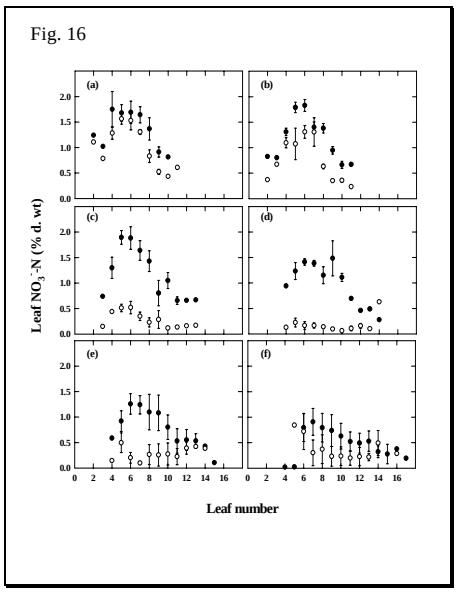


Fig. 16 Mean leaf NO_3^--N concentration (± SEM; n = 4) in *Lactuca sativa* after (a) 49 (b) 51 (c) 54 (d) 56 (e) 58 and (f) 61 d growth. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

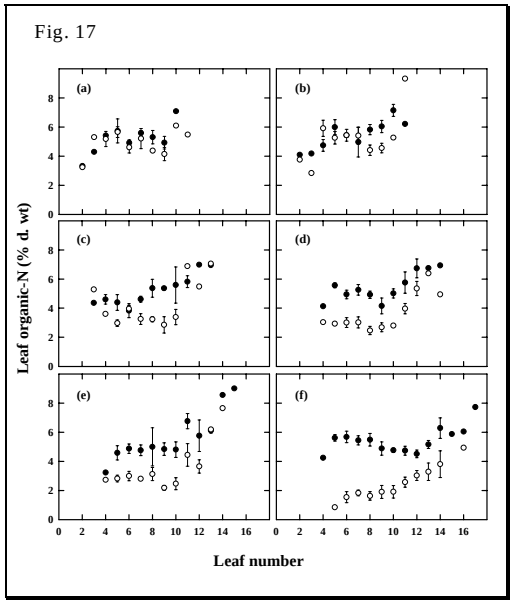


Fig. 17 Mean leaf organic-N concentration (± SEM; n = 4) in *Lactuca sativa* after (a) 49 (b) 51 (c) 54 (d) 56 (e) 58 and (f) 61 d growth. Filled circles, control plants. Open circles, N-limited plants, whose supply of N was removed on day 47.

The NO_3^--N (Fig. 16) and organic-N (Fig. 17) concentrations were recorded in all leaves at 2, 4, 7, 9, 11 and 14 d following imposition of treatments. In the control plants, NO_3^--N concentration was highest in intermediate leaves. Differences in leaf NO_3^--N concentration between the two treatments occurred after 2 d of N starvation, particularly in the younger leaves. After 4 d, the differences became pronounced, and after 7 d, virtually all NO_3^--N had disappeared in N-limited plants. However, leaf NO_3^--N concentration also appeared to decrease with time in all leaves in the control plants. Organic-N concentration was higher in new leaves than in older leaves. Differences in organic-N concentration between control and N-limited plants occurred in leaves 7-to-10 after 7 d. In younger leaves, there were no differences in organic-N between control and N-limited plants, indicating that even under severe N-deficiency, organic-N concentration is maintained in the new leaves.

Up to 80% of leaf organic-N is allocated to photosynthetic proteins and A_{max} and N concentration correlate strongly across a wide number of species (Field & Mooney, 1986; Evans & Seemann, 1989). Many other studies also show A_{max} to be highly correlated with the amount or activity of photosynthetic components such as rubisco, cytocrome-f and other coupling factors (Hikosaka & Terashima, 1995). It is therefore tempting to conclude that, since A_{max} and leaf organic-N are associated in our studies, that N-limited growth of plants is caused by a lack of photosynthetically-related enzymes. However, N-limited plants did not attain the same level of g_s as control plants and we conclude that g_s limits growth, at least in the early stages of N-deprivation. Two hypotheses describe how g_s may limit growth during N deficiency: (i) N supply affects the frequency or distribution of stomata, or (ii) N limits gas exchange across stomata. N supply did not affect the frequency or distribution of stomata in our studies. The second hypothesis, that N limits gas exchange across stomata, could be caused by increased CO_2 partial pressure at the sites of carboxylation causing compensatory

stomatal closure or via a direct signal. However, further work would be required to elucidate this process, for example by measuring *A* at different sub-stomatal CO_2 partial pressures ('A/C_i' curves) under N deficiency.

Measurements of epidermal cells indicate that the final size of young leaves in N-limited plants was reduced by cell division in early development, and cell expansion as the leaves mature (Figs 12-14). Therefore, in the leaves that form the bulk of the photosynthetic apparatus, cell expansion rate is the dominant growth component compromised during N deficiency. These data are consistent with data reported from sunflower (*Helianthus annuus*) (Trápani et al., 1999) and *Ricinus communis* (Roggatz et al., 1999). The distribution of NO_3^--N and organic-N in individual leaves suggests that the onset of N-deficient responses is associated with a loss of NO_3^--N in all leaves. Since the lack of cell and leaf expansion in N-limited plants will be caused by osmotic disruptions in the leaf, it is possible that NO_3^- is involved, either directly or indirectly, in this response. Nitrate may function as an osmolite (Cárdenas-Navarro et al., 1999) or as a signalling molecule (Zhang & Forde, 2000) in plants. In tomato, leaf-growth declines in response to N-stress before reductions in total plant weight gains or photosynthetic potential, possibly through the production of ABA which can cause stomatal closure (Chapin, 1990). Further strategic work could determine if g_s responds directly to nutrient-induced signals, or by compensating for lower rates of carboxylation.

2.3 Summary of Objectives 1-3

- An objective modelling technique accounted for 99.0 and 99.1% of the variation in plant dry weight for control and N-limited plants respectively. For P and K experiments, fits accounted for more than 97 % of the variation in both treatments.
- Sub-linear relationships occurred between nutrients and RGR under restricted nutrient supply conditions. These conform to a four-parameter dilution model.
- There were effects of all nutrient treatments on morphological and physiological components of growth and we focus in detail on the components of growth impacted by N supply.
- Leaf weight ratio (LWR) increased over time in control plants and decreased in N-limited plants and shoot:root ratio followed a similar pattern.
- On a whole-plant basis, assimilation of C decreased in N-limited plants, a response paralleled by differences in stomatal conductance.
- N-limited plants had lower light-saturated rates of photosynthesis on a leaf area basis (A_{max}), and no effect on photosynthetic efficiency (K_m) or light compensation point (I_{min}).
- The relationship between N and A_{max} paralleled the relationship between N and RGR; A_{max} approached zero at 2% N in the dry matter (Fig. 4).
- Although N-limited plants had lower maximum rates of C assimilation (*A*), comparisons at equivalent stomatal conductance values (g_s) showed that *A* was not directly limited by organic-N but by g_s, at least in the early stages of N deficiency.
- Reductions in g_s under N-limiting conditions did not associate with adjustments to stomatal frequency or distribution.
- Plants may respond directly to a lack of N, or through an indirect compensatory response to lower rates of carboxylation and thus an increased partial pressure of CO_2 at these sites.

2.4 Knowledge / technology tranfer from meeting Objectives 1-3

- We have published refereed papers on N (Broadley et al., 2000, 2001a), P (Broadley et al., 2002a), and K / replacement cation dynamics (White & Broadley, 2000; Broadley et al., 2001b).
- We have provided information to support the development of model-based lettuce decision support systems. Specifically, we have supplied data and ideas into DEFRA project to develop LET_N (HH1414SFV, 2001-2004). We have also supplied primary data to Ido Seginor (Agricultural Engineering Department, Technion, Haifa 32000, Israel) who is developing models of lettuce growth in Mediterranean climates.
- Since responses in growth are observed under N, P and K deprivation, we are using genomics-based approaches to test the hypothesis that nutrient deprivation is a centralised stress response, mediated by similar signal transduction cascades (we have secured funding from DEFRA, and from an HRI Browning Studentship to conduct this work).
- Methods to study P nutrition, developed in this project (Broadley et al., 2002a) are being used to characterise P-responsive 'Smart Plants' (HRI Browning Studentship, 2000-2003).
- Methods to study K nutrition, developed in this project (e.g. Broadley et al. 2001b) are being used to characterise ion transport in plants lacking specific genes encoding K transport proteins (BBSRC Committee Studentship, 2001-2004, HRI / University of Birmingham Studentship 2001-2004).
- Knowledge on the behaviour of replacement ions supported a study to provide policy advice on the movement of Cl^- in the food chain, for the Food Standards Agency (Philip White & Martin Broadley, 2000).
- An invitation to present strategic work on N nutrition in lettuce, delivered within this project, led to Ian Burns being asked to become the UK representative on the International Plant Nutrition Committee.

3 RELATIONSHIPS BETWEEN NUTRIENT SUPPLY AND WHOLE-PLANT GROWTH THROUGH THE LIFETIME OF THE CROP.

To meet Scientific Objective 4, experiments were conducted on different crop types, grown to maturity in a nutrient-film technique (NFT) hydroponic system. Plants were grown under either nutrient-replete, or nutrient deficient (lacking one nutrient) conditions by withholding N during plant vegetative growth. Nitrogen was removed at different times of growth. Measurements of growth were compared with a control treatment in which all N was supplied continuously. Aspects of the work have been submitted for publication (Broadley et al., 200*). The generic methods are outlined and detailed results presented for the butterhead lettuce crop.

3.1 Materials & Methods

3.1.1 Plant material

Experiments were performed on four crop types, butterhead lettuce plants (*Lactuca sativa* L. cv. Kennedy), supplied as pelleted seed (Elsoms Seeds Ltd, Spalding, Lincs., UK), cauliflower (*Brassica oleracea* var. *botrytis* L. cv. Fandango), dwarf French bean (*Phaseolus vulgaris* L. cv. Newton) and spinach (*Spinacia oleracea* L. cv. Spinnaker). Crops were sown in rockwool blocks (3.5 cm x 3.5 cm x 4 cm; Grodan, Hedehusene, Denmark) and watered with tap water in plastic trays. After germination, the seedlings were watered with a full strength nutrient solution (Table 1). At 14 days after sowing (DAS) the plants were transplanted in their individual rockwool blocks to the NFT system.

3.1.2 NFT System

The NFT system comprised 12 individual gullies (5.15 m length x 0.11 m width x 0.05 m depth) constructed from flat-bottomed PVC guttering. The gullies were spaced 0.26 m apart (centre-to-centre) in two groups of six within the same glasshouse compartment. Seventy-two holes, each of a sufficient size to contain a single rockwool block (diameter 4.5 cm), were cut at equal distances (every 6 cm) along 4.32 m strips of PVC. One of these strips was secured to the top of each gully and the remaining 0.83 m of guttering was covered with a separate strip of PVC containing no holes. A single rockwool block was placed in each hole so that the base rested directly on the bottom of the gully. Each gully was connected to two water-storage tanks that each contained 200 L of nutrient solution. Taps were used to control which tank supplied which gully at any given time. This allowed for different N treatments to be imposed during the experiment. In all, three different N treatments were imposed: (i) nutrient solution supplied continually (control, T1), (ii) N withdrawn during early vegetative growth (T2) and (iii) N withdrawn in late vegetative growth for lettuce and spinach, during curd formation in cauliflower, and during pod-filling in French bean (T3). In the -N solution NH_4NO_3 and $Ca(NO_3)_2.2H_2O$ were replaced by $CaSO_4.H_2O$ (Table 1). The nutrient solutions were pumped from the supply tanks to the top-end of the gullies. Subsequently, the nutrient solutions flowed along the bottom of the gullies under the influence of gravity (the gradient was < 2°) and drained back into the tanks. The flow rates were controlled using taps, to ensure that the nutrient solutions flowed in a thin layer (c. 2 mm in depth) along the bottom of each gully. During the experiment, individual plants were sampled, and the resulting gaps in the PVC strips were immediately covered to maintain humidity and to prevent algal growth within the gullies.

Fig. 18 Nutrient Film Technique (NFT) system used to grow field vegetable crops to maturity. Crops shown are cauliflower (top left and bottom right), spinach (top right) and butterhead lettuce (bottom left).

3.1.3 Experimental design

The experiment was designed so that the 12 gullies (each running along an east - west axis) were arranged into four units (along the north - south axis). Thus, each unit contained three gullies, one for each of the three treatments, randomly placed within each unit. Each gully had 72 individual holes (positions for plants). Four sections were imposed along each gully, each consisting of 18 plants. At each sampling date, one plant was taken from each section of each gully and the four plants from each gully bulked for analyses. At each sampling date, one plant was taken from each section of each gully and the four plants from each gully bulked for analyses. Overall, therefore, the experiment was designed so that any effect of the north - south gradient could be removed, and that the variation over the east - west axis was minimised. Analyses of variance were performed to assess the effects of treatment and of sampling date within treatment assuming a split-plot design: treatments applied to main plots whilst sampling date applied to subplots (plants within tanks). All statistical analyses were performed using Genstat (Fifth Edition, Release 4.2, VSN International, Oxford, UK).

3.1.4 Plant measurements

Plants were sampled destructively. The following measurements were taken: shoot fresh and dry weights, shoot nitrate-N, shoot total-N and shoot total-C concentation. Shoot dry weights were used to estimate the absolute growth rates (AGR) and the relative growth rates (RGR) of plants, using the RSCHNUTE procedure in Genstat (see Section 2). For the butterhead lettuce, growth was modelled as a function of cumulative day-degrees (ΣEDD), assuming a light efficiency factor of 0.1 and a base temperature of 0 °C (Scaife et al., 1987). Nitrate-N was measured using the extraction procedure of Hunt and Seymour (1985). Briefly, a maximum of 0.2 g sub-sample of homogeneous, milled, dry leaf tissue was shaken for 30 min in a flask containing 50 ml deionised H_2O and 200-300 mg of activated charcoal. The solution was filtered through Whatman No. 1 filter paper, with the initial 2 ml of filtrate discarded. The filtrate was analysed for nitrate-N using a continuous flow colormetric method on a flow-injection analyser (FIASTAR 5012, FOSS Tecator, Sweden). If the dry leaf weight was less than 0.05 g, the amount of deionised water used was adjusted; for samples less than 0.05 g 25 ml of deionised water was added and for samples less than 0.02 g 10ml of water was added. Total-N and total-C were measured directly on 0.5-1 g of dried and milled plant material using a C:N analyser (CN2000, LECO, Stockport, UK). Organic-N represents the difference between total-N and nitrate-N. For the first three harvests, plant material from three gullies were bulked before mineral analyses were performed.

3.2 Results & Discussion

The mean temperature and light levels declined gradually during the course of the lettuce experiment (Fig. 19). The fresh and dry weights of lettuce shoots increased as a function of cumulative physiological time in all treatments, although increases were slower in plants once N supply was removed (Fig. 20). Richards' and Gompertz growth functions were selected automatically by the RSCHNUTE procedure for the three treatments (Table 2). These fits accounted for almost all of the variation in shoot growth in all treatments (r^2 > 0.985, d.f. = 1, 15), and fitted shoot dry weights are presented (Fig. 20). The changes in shoot AGR and RGR as a function of cumulative physiological time differed as a result of treatment (Fig. 21). The AGR of T2 plants remained constant over > 200 EDD units before declining. The AGR of T3 plants increased following the removal of N supply, albeit at a slower rate than T1 plants. Thus, the selected growth curves gave an excellent fit to shoot dry weight data. The effectiveness of this procedure to estimate RGR of plant shoots is consistent with studies outlined in Section 2 to estimate RGR of whole-plants. However, these are the first RGR data estimated as a function of physiological time using this procedure.

Physiological changes that corellate with this reduction in shoot growth under N limiting conditions are a rapid increase in % d. wt and a rapid reduction in % C on a dry weight basis (Fig. 22). Plants experiencing N-stress decrease leaf weight ratios, reallocate C from shoots to roots, and reduce C assimilation rates, either through enzyme limitation or through a direct reduction in stomatal conductance. Since the percentage dry weight increased and the proportion of C in the dry weight decreased under N-limitation, our study is consistent with recent studies on other crops such as tomato (Le Bot et al., 2001). However, it is not possible to identify the precise causes of growth reductions under limited N supply from whole-plant studies.

Fig. 19

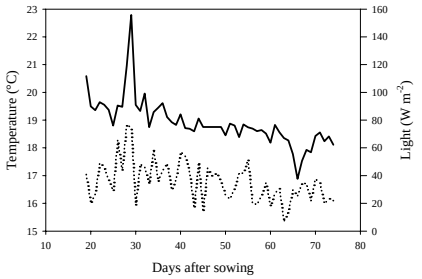


Fig. 19 Daily temperature and light levels (mean levels per 24 h), from 19 days after sowing.

Fig. 20

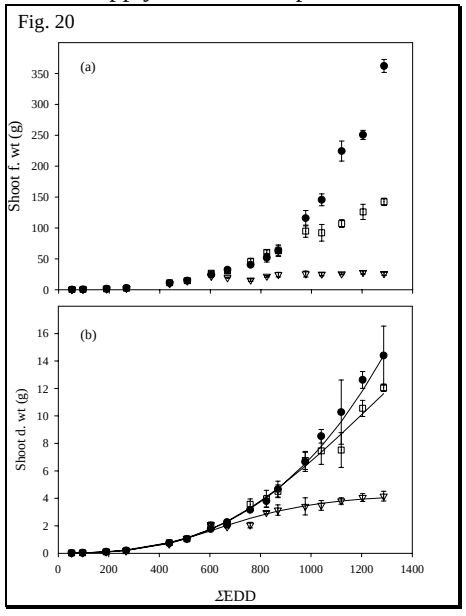


Fig. 20 Shoot fresh (a) and dry (b) weights of *Lactuca sativa* (mean ± S.E.M; n = 4). Filled circles, control plants (T1). Open symbols, N-limited plants, whose external N supply was removed 35 (triangles, T2) and 54 (squares, T3) days after sowing. Fitted dry weights are Gompertz (T1 and T3) and Richards' (T2) functions obtained using RSCHNUTE in Genstat.

Fig. 21

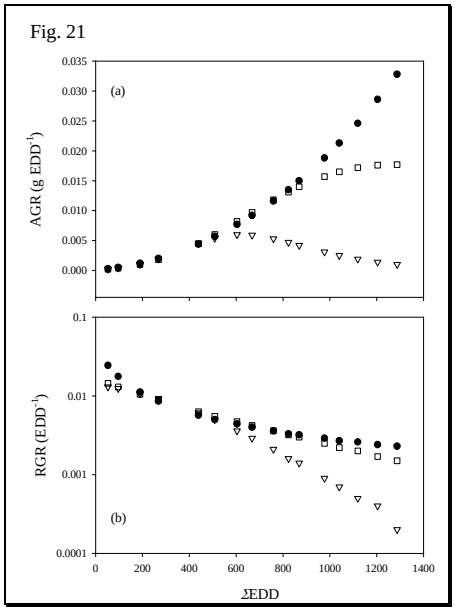
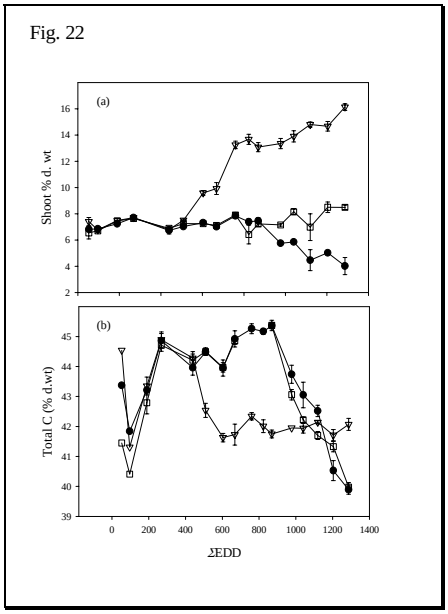
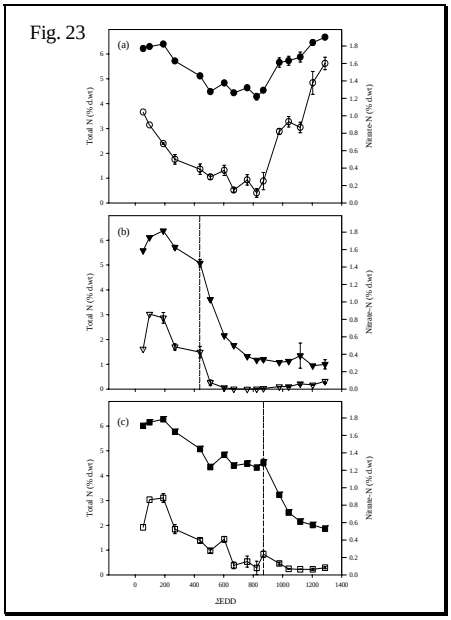


Fig. 21 (a) Absolute (AGR) and (b) relative (RGR) growth rates of *Lactuca sativa* shoots as functions of cumulative physiological time. Filled circles, control plants (T1). Open symbols, N-limited plants, whose external N supply was removed on 35 (triangles, T2) and 54 (squares, T3) days after sowing. Rates are estiamted using Gompertz (T1 and T3) and Richards' (T2) growth functions using RSCHNUTE in Genstat.

In control plants, there was an ontogenetic decline in total-N and nitrate-N until the plants had grown for 800 EDD units (Fig. 23a). This decline in shoot N occurs because as plants grow in size, structural and storage materials, containing little N, will increase in volume faster than the size of photosynthetically-active surfaces, due to scaling constraints (Caloin & Yu, 1984; Hardwick, 1987; Greenwood et al., 1990). However, in the latter stages of growth, there was a rapid increase in nitrate-N levels in control plants, and a slight increase in organic-N. In T2 plants, a rapid and almost complete loss of shoot nitrate-N followed the removal of N supply (Fig. 23b). This was parallelled by a decline in total-N to an asymptotic minimum concentration of c. 1 % d. wt, although the decline in total-N was less rapid than for nitrate-N. In T3 plants, the nitrate-N levels had increased slightly (in parallel with T1 plants) when the -N treatment was imposed (Fig 23c). However, there was minimal nitrate-N in the plant at this time, and nitrate-N continued to decline after this point. The total-N declined following the removal of N in T3 plants, but not to an asymptote of 1 % as observed in T2.





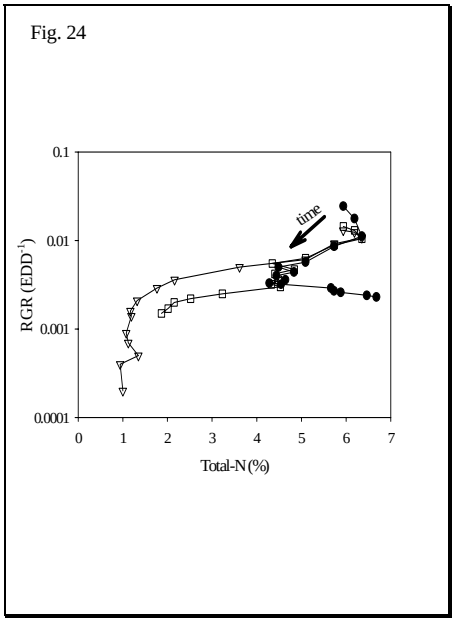


Fig. 22 Physiological changes in *Lactuca sativa* (cv. Kennedy) as functions of cumulative physiological time. (a) Shoot dry tissue concentration and (b) shoot C concentration as a proportion of d. wt (mean ± s.e.m.; n = 4). Filled circles, control plants (T1). Open symbols, N-limited plants, whose external N supply was removed on 35 (triangles, T2) and 54 (squares, T3) days after sowing.

Fig. 23 Nitrogen dynamics in the shoots of *Lactuca sativa* (cv. Kennedy) as functions of cumulative physiological time. (a) Control plants (T1), (b) N-limited plants, whose external N supply was removed 35 days after sowing (T2), (c) N-limited plants, whose external N supply was removed 54 days after sowing (T3). Filled symbols, total-N, open symbols, nitrate-N.

Fig. 24 Relative growth rate (RGR) of dry *Lactuca sativa* (cv. Kennedy) shoots as a function of total-N concentration. Filled circles, control plants (T1), open symbols, N-limited plants, whose external N supply was removed on 35 (triangles, T2) and 54 (squares, T3) days after sowing. RGR was derived from fits of Gompertz (T1 and T3) and Richards' (T2) growth functions using the RSCHNUTE procedure in Genstat. The time direction is indicated by an arrow.

In both sets of N-limited plants, the supply of N was withdrawn before self-shading resulted in an increase in shoot nitrate concentration. The amount of N in the shoots remained constant following the removal of external N supply in both sets of N-limited plants. Thus, no significant amounts of N were reallocated to roots, or lost from the plant. The decline in shoot nitrate-N concentration was more rapid than the decline in shoot organic-N concentration. This result is consistent with studies on crisphead lettuce; when plants are forced to rely on the utilization of internal reserves for growth it is more feasible for plants to utilize nitrate-N than to recycle organic forms (see Walker et al., 2001 for more details). In control plants, when leaves are shaded, rates of photosynthesis decline, and this reduces the requirement for nitrate reduction. This can result in the luxury accumulation of N, in particular nitrate-N, in plant tissues. Thus, the increase in shoot nitrate-N in control plants is due to self-shading as the plants begin to heart and reach commercial maturity. The effect of self-shading is compounded by the gradual reduction in light and temperature levels experienced by the plants during this experiment. The accumulation of nitrate-N in leafy salad crops is of great concern to growers of salad crops since there are limits on the permitted tissue nitrate levels in Europe. The propensity of crops such as butterhead lettuce to accumulate nitrate are of particular concern to growers of this crop in Northern Europe, in particular under glass, where light levels are low (Escobar-Gutiérrez et al., 2002).

Relative growth rate declined with physiological time in all treatments (Fig 24). As a function of total-N, RGR increased in a sub-linear manner in T2 and T3 plants. A four parameter model which assumes that the decline in shoot RGR after an interruption in N supply is a linear function of the reciprocal of the total-N concentration of the shoot, and that the redistribution of N from shoots to roots is minimal, was fitted to data (Equation 2). In plants from both of the N-limited treatments, the RGR versus shoot N concentration relationship fitted the model (T2, $r^2 = 0.923$; T3, $r^2 = 0.982$). Thus, these results are consistent with studies in crisphead lettuce outlined in Section 2.

The ontogenetic effect of a decline in RGR with plant age could be removed by calculating the RGR as a proportion of the control RGR for the same harvest ($RGR / RGR_{control}$). The effects of total-N concentration on proportional RGR were similar in T2 and T3 plants, although in T3 plants, the N concentrations and proportional RGR did not decline to the same level as T2 plants (Fig. 25a). A similar effect was observed for the effects of organic-N on proportional RGR (Fig. 25b). Proportional RGR was largely independent of nitrate-N concentration of the plants (Fig. 25c). Control data are presented in the same figure for comparison (Fig. 25a-c). In addition to an ontogenetic decline in RGR with plant age, there is an age-related increase in both total-N and organic-N in control plants during the latter stages of crop growth (see Fig. 23). The simultaneous ontogenetic effects of declining RGR and increasing N

concentration can be removed by calculating RGR and the total-N and organic-N data as proportions of those in control plants taken at the same harvest. The effects of total-N and organic-N on RGR, expressed as proportions of N concentration and RGR of the control data, are similar for T2 and T3 plants (Fig. 26). Thus, the proportional effects of total-N and organic-N on plant RGR were independent of plant age.

Fig. 25

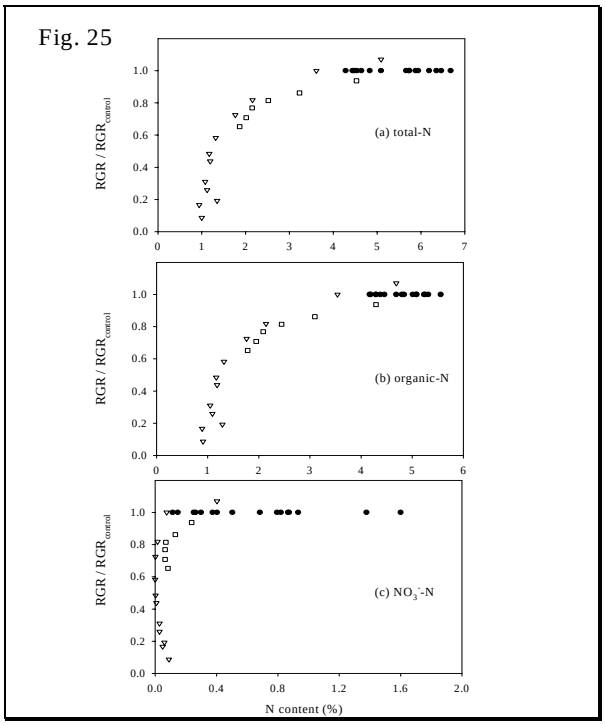


Fig. 25 Relative growth rates (RGR), expressed as a proportion of control RGR, as functions of: (a) the total-N concentration (b) the organic-N concentration and (c) the nitrate-N concentration of dry *Lactuca sativa* (cv. Kennedy) shoots. Filled circles, control plants (T1). Open symbols, N-limited plants, whose external N supply was removed 35 (triangles, T2) and 54 (squares, T3) days after sowing.

Fig. 26

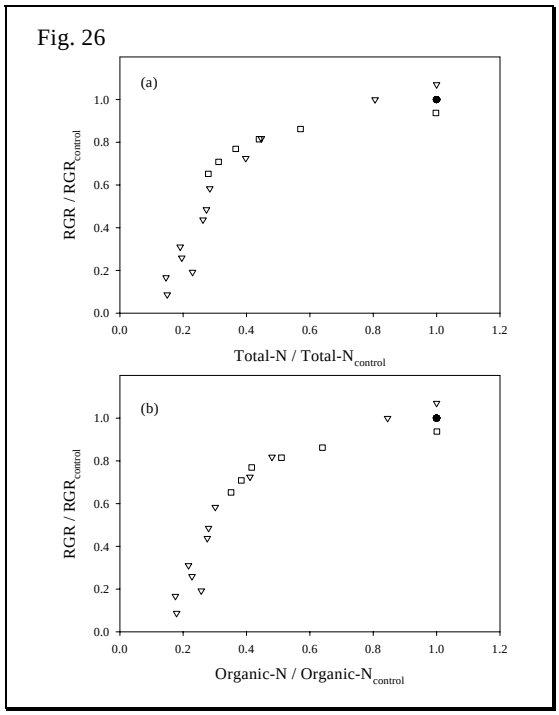


Fig. 26 Relative growth rates (RGR), expressed as a proportion of control RGR, as functions of (a) total-N concentration and (b) organic-N concentration of dry *Lactuca sativa* (cv. Kennedy) shoots. Total-N and organic-N concentration are expressed as proportions of control values. Filled circles, control plants (T1). Open symbols, N-limited plants, whose external N supply was removed 35 (triangles, T2) and 54 (squares, T3) days after sowing.

Fig. 27 Nitrogen deficiency symptoms in NFT experiments. From left to right cauliflower, butterhead lettuce (from bottom to top T1, T3 and T2), dwarf French bean (T2 plants in the foreground) and spinach (bottom left).

In control plants, the ontogenetic increase in shoot accumulation of luxury N meant that previously observed linear relationships between N and RGR (Section 2) were not observed. This is likely to be due to two factors. First, unlike previous studies, control plants were grown to commercial maturity, and thus self-shading was maximised. Second, butterhead varieties lettuce have a higher N concentration, in particular nitrate-N, than crisphead varieties (Escobar-Gurtiérrez et al., 2002). Therefore it is important to take both genotypic and ontogenetic factors into account when modelling the N dynamics of lettuce and for designing assays to screen for NUE, or low-nitrate concentration, even within single crop species.

Although, nutrient interruption techniques in hydroponics do not equate to field-conditions, soil-grown plants are likely to experience periods where they are forced to rely on internal redistribution of nutrients and therefore these techniques have a general merit for understanding. This study has determined that there is a positive sub-linear relationship between RGR and N concentration in butterhead lettuce, and has demonstrated that the proportional effects of this relationship are independent of plant age. Thus, periods of N deprivation can still affect crop yields in latter stages of crop growth, although this effect will become smaller as the plants get closer to maturity. In addition to providing data to underpin models of the response of lettuce to N fertilisers, these techniques show that the design of assays to screen for NUE or nitrate-N concentration of different genotypes of lettuce depend critically on both the conditions in which the plants are grown and the age of the plant. It will be important to design screens appropriate to the environment in which the crop will be grown, and according to how nutrients will be supplied.

3.3 Summary

- Almost all variation in shoot growth, as a function of 'physiological time', was explained using our chosen growth models.
- We used a four-parameter dilution model to predict shoot RGR as a function of shoot N concentration, to which the data gave excellent fits.
- The proportional effects of total-N and organic-N on shoot RGR were independent of plant age.
- This is the first use of 'physiological time' to estimate RGR using an objective model-fitting technique.
- The results from a whole-crop, grown to commercial maturity, are consistent with more detailed studies in Section 2, and thus supports the use of strategic studies to understand constraints to nutrient efficiency at the crop level.
- We can use these data to design assays to screen for nutrient-use efficiency traits in crop genetic resource collections.
- It is important to design assays to screen for nutrient-use efficiency traits that are appropriate to the environment in which the crop will be grown, and according to how nutrients will be supplied.

3.4 Knowledge / technology tranfer from meeting Objective 4

- We have submitted a paper on the response of butterhead lettuce to N (March 2002)
- We have supplied data and ideas to Ido Seginor (Agricultural Engineering Department, Technion, Haifa 32000, Israel) who is developing models of lettuce-growth in Mediterranean climates.
- We have supplied data and ideas to DEFRA LINK project (LK0438) to develop LET_N, a model-based decision support system for use by the commercial protected lettuce industry.
- We have designed assays for a more applied DEFRA project on P nutrition.
- We have discussed our ideas with lettuce breeding experts, and we are to submit a concept note to DEFRA to screen for desirable traits in lettuce and spinach.

4 TO IDENTIFY GENETIC LOCI IMPACTING ON P-USE EFFICIENCY IN MODEL BRASSICACEAE

4.1 Introduction

To meet Scientific Objective 5, a series of experiments were conducted on model Brassicaceae (cabbage family) species. In the first series of experiments, *Arabidopsis thaliana* (L.) Heynh (thale cress) was used and in the second series of experiments, *Brassica oleracea* L. (cabbage and its allies) was used. This component of the project was designed to explot natural genetic variation to obtain preliminary strategic information on the genetics of P-use efficiency (PUE; defined as the yield per unit input of P) in plants. Phosphorus was selected as an appropriate nutrient to work on since it is the macronutrient used least efficiently by crops and it is potentially a serious environmental contaminant.

4.2 Natural variation in P use efficiency in *Arabidopsis thaliana*

Arabidopsis is an ideal plant for performing genetic studies. This is because (1) it has a small, fully sequenced genome, (2) a large number of available mutants, and (3) there is considerable natural genetic variation in *Arabidopsis* since it has a wide natural geographical distribution (Alonso-Blanco & Koornneef, 2000). We aimed to exploit the natural genetic variation in *Arabidopsis*. Molecular markers, distributed evenly across all linkage groups, can be identified that are polymorphic between different ecotypes. Ecotypes can subsequently be crossed (Lister & Dean, 1993). The progeny are then immortalised by single seed decent to produce recombinant inbred lines (RILs) that are homozygous at each locus. Phenotypic traits can be scored in RILs, and the contribution of different markers to the trait can therefore be estimated. The contribution of specific markers can be used to identify likely genetic loci impacting on the trait in question (quantitative trait loci, or QTL). RILs offer specific advantages because of the ability to repeat an experiment almost indefinitely and in different environments. At present, there are three available mapping populations of *Arabidopsis*, including Landsberg *erecta* (L*er*-k, N8581) x Cape Verdi Island (Cvi, N8580) (Alonso-Blanco et al., 1998), Niederzenz (Nd-1, N1636) x Columbia (Col-3, N908 and Col-5, N1644) (Holub & Beynon, 1997; Deslandes et al., 1998) and Landsberg *erecta* (Ler-0, NW20) x Columbia (Col-4, N933) (Lister & Dean, 1993). These populations have been used to study quantitiative traits such

as flowering time (Alonso-Blanco et al., 1998; El-Assal et al., 2001), circadian leaf movements (Swarup et al., 1999) and seed germination (van der Schaar et al., 1997). Recently, we have used the L*er* x Cvi population to identify a locus impacting on cation accumulation (White et al., 2002). Since PUE is likely to behave as a quantitiative genetic trait, we adopted this approach to investigate the genetics of PUE in *Arabidopsis*.

4.2.1 Materials & Methods

4.2.1.1 Plant Material

Five accessions of *Arabidopsis thaliana* were chosen to screen for PUE, based on previously observed variation in phosphate acquisition efficiency (PAE; Narrang et al., 2000): Cal and Col-0 were selected to represent accessions with low PAE, C24 with high PAE, and Ws-2 and Ler-0 with intermediate values of PAE. A further four accessions of *Arabidopsis* were also selected for screening: Landsberg *erecta* (L*er*-k), Cape Verdi Island (Cvi), Columbia (Col-5) and Niederzenz (Nd-1). These accessions represent parents of available mapping populations of RILs. Further, Col-5 has been used by White and Rahn (1999) as a background accession to generate transgenic lines expressing GFP or GUS fused with a promoter for a phosphate sensitive gene *SQD1* (Essigmann et al., 1998). Seeds of all nine accessions were obtained from the Nottingham Arabidopsis Stock Centre, UK (NASC).

4.2.1.2 Growth Conditions

Seeds of *Arabidopsis* were imbibed in de-ionised H_2O for 2-5 days at 4 ºC to break dormancy. Plastic seed trays, divided into cells (9 mm width x 9 mm length x 28 mm depth) were supported in larger plastic trays (whose dimensions varied according to experiment) and filled with dried silica sand (Hepworth Minerals and Chemicals, Cheshire, UK). Imbibed seeds were sown into each cell. Trays were irrigated with nutrient solution (pH = 5.6) containing: KOH (0.50 mM), $MgSO_4.7H_2O$ (0.75 mM), $CaCl_2.2H_2O$ (0.03 mM), FeNaEDTA (0.10 mM), $Ca(NO_3)_2.4H_2O$ (4.00 mM), H_3BO_3 (30.0 µM), $MnSO_4.4H_2O$ (10.0 µM), $ZnSO_4.7H_2O$ (1.0 µM), $CuSO_4.5H_2O$ (3.0 µM), and $Na_2MoO_4.2H_2O$ (0.5 µM). Appropriate quantities of KH_2PO_4 and KH_2SO_4 were added to alter the phosphate concentrations of the nutrient solutions, whilst maintaining the levels of K. With the exception of one experimental block, the nutrient solutions were radioactively labelled with 256 kBq $KH_2{}^{33}PO_4$ L^{-1} (specific activity >148 TBq $mmol^{-1}$, Amersham Pharmica Biotech, Buckinghamshire, UK). Plants were grown in a Saxcil growth cabinet (S.K. Saxton Ltd., ARC Works, Cheshire, UK) for 21 days under a 16 h photoperiod, at 24 °C day and 16°C night. Light intensity was approximately 75 µmol m^{-2} s^{-1} at plant height and relative humidity was set to 80%. Glass lids were placed over the tray for the first week to maintain humidity, then removed. Trays were watered with 50 mL of de-ionised H_2O every 3-4 days.

4.2.1.3 Plant measurements

In all experiments, the shoots of the plants were harvested and weighed 21 d after sowing. Plant shoots were placed in plastic scintillation vials and 5 mL of Ecoscint A was added (National Diagnostics, Hull, UK). Samples were counted using a Beckman LS6000TA liquid scintillation system (Beckman Instruments Inc., California, USA). Tissue P was estimated using counts per minute (cpm) from individual plants using Equation 3.

$$\text{Internal P (µg P g}^{-1}\text{ f. wt)} = \left(\text{Plant activity (cpm)} \times \left(\frac{\text{µg P in solution}}{\text{solution activity (cpm)}} \right) \right) \Big/ \text{plant f. wt (g)} \qquad \text{Equation 3}$$

In the one experimental block where ^{33}P was not used, shoot tissue was ashed at 490 °C for 14-16, and 1 mL of concentrated HNO_3 and 5 mL of H_2O was subsequently added. Total shoot P was determined on filtered extracts, as descibed in Section 3. Phosphorus-use efficiency was calculated as shoot fresh weight divided by P concentration in the shoot.

4.2.1.4 Experiment 1: screening At accessions for PUE

All nine accessions of *Arabidopsis* were used. Plastic seed trays were filled with silica sand, as described above. Seed trays were supported within a PVC tray comprising eight independent channels. Two channels were used for each experimental block and each block was separated by an empty channel. Each channel was subdivided into nine sections, one for each accession, comprising 16 cells in each section. The sections were randomly oriented within each block. Three seeds of each accession were sown into appropriate cells. One channel in each block received 250 mL nutrient solution containing 625 µM P, and the other received 250 mL nutrient solution containing 62.5 µM P. The experiment was repeated on two occasions. Analyses of variance were used to test for differences in PUE between accessions using using Genstat (Fifth Edition, Release 4.2, VSN International, Oxford, UK).

4.2.1.5 Experiment 2: Quantifying genetic variation in PUE

A population of 162 RIL from a cross between L*er* and Cvi was obtained from NASC. These RIL contain 99 molecular markers spanning all five chromosomes (Alonso-Blanco et al., 1998). Plastic seed trays were filled with silica sand, as described above. The plastic seed trays were supported within a single-compartment plastic tray. Three seeds of the same RIL were sown into each cell; two cells were sown for each RIL, and for the parent lines, L*er* and Cvi. Cells were randomly selected for each of the lines sown. Each seed tray (consisting of 328 sown cells) received 700 mL of nutrient solution containing 62.5 µM P. The experiment was repeated three times. In the first run ^{33}P was not used and the plants were analysed by ICP-ES (see Section 3). QTL analyses were

performed for each experimental run using the interval mapping option of the MapQTL programme (van Ooijen & Maliepaard, 1996). This determines the likelihood of there being a QTL versus no QTL between pairs of markers (LOD score). A LOD score in excess of 2 can be taken as a suggestive QTL. Significant QTL ($P < 0.05$) require a LOD score of 2.8 (van Ooijen, 1999).

4.2.2 Results & Discussion

PUE varied four-fold between accessions at low P. C24 had, by far, the best PUE at low P and Cal had the best PUE at high P. Col-0 had the worst PUE at both low and high P. This conforms to the results of Narang et al. (2000). There was significant differences in PUE between L*er* and Cvi at high P, but not a low P (Fig. 28). In the three experimental qtl runs, 149, 159 and 87 RIL were analysed respectively. This number varied due to uneven growth and germination. The frequency distributions of growth (shoot f. wt), P concentration and PUE were normally distributed in each experimental run (Fig. 29). Mean values of the parent lines are indicated on the figures; transgressive segregation of traits was observed on all traits in all experimental runs. L*er* had lower shoot f. wts and PUE than Cvi. There was no difference in shoot P concentration between ecotypes. Significant, or suggestive, QTLs were observed within the top 25 cM of Chromosome I for shoot f. wt in experimental runs 1 and 3. These coincided with significant QTLs for PUE at this locus (Fig 30). There was also a suggestive QTL for shoot f. wt and PUE at approximately 40 - 50 cM along Chromosome IV (not shown). This was observed in experimental runs 1 and 2. Within each of the runs, up to 30% of the variation in PUE was genetic.

The identification of significant and suggestive QTL impacting on plant growth and PUE indicates that there is significant genetic component to these traits. The observation of a significant QTL impacting on shoot f. wt within the top 25 cM of chromosome I corresponds to other experiments that we have performed using sand-grown L*er* and Cvi (White et al., 2002). Although we have identified a large genetic effect on plant growth and PUE in *Arabidopsis*, there is also a large environmental (and presumably genotype X environment) effect impacting on these traits. It was not possible to resolve these components in this study, since there was large plant-to-plant variation between experimental runs. Since experimental conditions were standardised and controlled within the growth cabinet, the most parsimonious explanation for the large plant-to-plant variation between experimental runs is that the sand was not a sufficiently homogeneous substrate. To further study the genetic components of mineral nutrition in small herbaceous plants such as *Arabidopsis*, we recommend that the next series of experiments are performed in a homogeneous substrate, for example in hydroponics, or ideally in nutrient agar. A preliminary study in nutrient agar has indicated that plant-to-plant variation in nutrient-efficiency traits can be identified rapidly and minimised in this medium.

Loci impacting on shoot growth and PUE can be resolved through fine mapping. Resources are available to do this work, such as near isogenic lines and/or mutants in appropriate background lines (Swarup et al., 1999) which can be introgressed to map genes. The success of this technique has recently been proven for the first time in *Arabidopsis* where a locus, coincidently at the top of Chromosome I, that impacts on flowering time has been fine mapped to a single gene, and even to a single amino acid substitution (El-Assal et al., 2001). Fine-mapping genes in *Arabidopsis* allows precise molecular markers to be assigned to specific traits. By determining regions of synteny (conservation of gene order) between *Arabidopsis* and crop species, markers can be used to screen crop plants for desired traits and this informaton could be used in breeding programs. The sequencing and physical mapping of several crop plant genomes will be complete in the next few years. This will allow molecular markers assigned to specific traits in *Arabidopsis* to be associated with precise loci of economically-important species. Since nutrient-use efficiency is a fundamental trait of huge commercial importance for crop growth, we recommend that the genetic components of this trait are resolved in *Arabidopsis* and that now is the time to do so.

Fig. 28

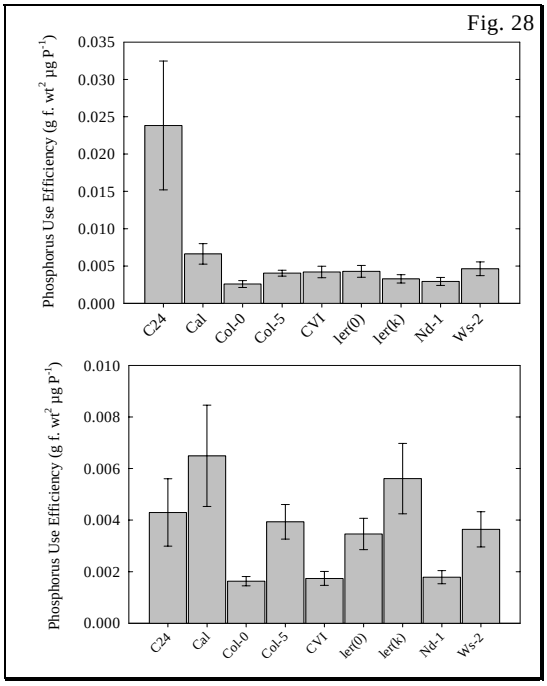


Fig. 28 Phosphorus use efficiency of *A. thaliana* accessions. Accessions were grown for 21 days at low (top panel, 62.5 µM) or high (lower panel, 625 µM) phosphate concentrations on fine silica sand (mean ± SEM; n > 20 plants).

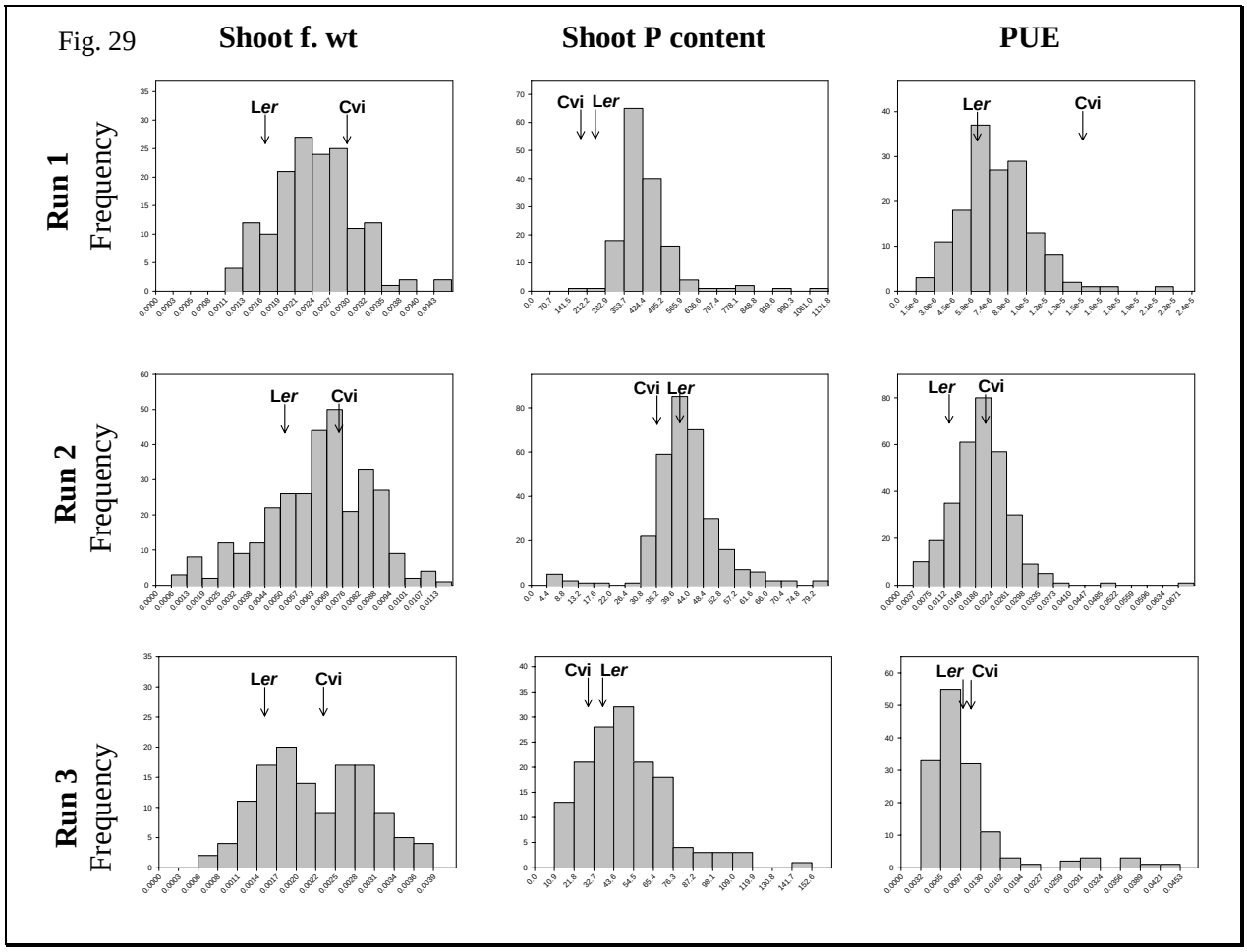


Fig. 29 . The frequency distributions of shoot fresh weight, shoot P concentration and P-use efficiency traits of 162 recombinant inbred (RI) lines of *A. thaliana* from a L*er* x Cvi cross. Plants were grown for 21 days in fine silica sand supplied with a nutrient solution containing 62.5 µM phosphate radiolabelled with ^{33}P. Trait values of parent lines are indicated. Data from all three runs are presented.

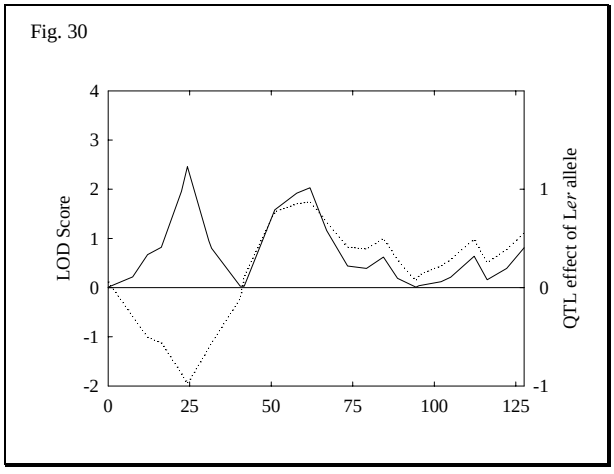


Fig. 30 QTL for P-use efficiency on Chromosome 1. The QTL likelihood (LOD score) and QTL effect were obtained using the MQM procedure of MapQTL. Solid lines indicate LOD score and broken lines indicate the QTL effect of the L*er* allele.

4.3 Natural variation in P use efficiency in *Brassica oleracea*

In addition to studies on *Arabidopsis*, it is possible to characterise the genetic components of PUE, and perform QTL analyses, directly in crop plants where appropriate mapping populations are available. We have conducted preliminary proof-of-concept studies to characterise the genetic components of PUE in a mapping population of doubled-haploid *Brassica oleracea*, a close relative of *Arabidopsis* containing many important commercial crop species.

4.3.1 Materials & Methods

We adopted a QTL approach to study PUE in *Brassica oleracea.* We used the 'NB' population of doubled haploid lines arising from a cross between two DH parents, a cauliflower (*Brassica oleracea* L. var. *botrytis* L.) line and a calabrese inbred line (*Brassica oleracea* L. var. *italica* Plenck), through microspore culture of the the F_1 (GJ King, unpublished observations). Six seeds of each accession were sown; three seeds to an 11cm square pot filled with Levington M2 peat based compost (Scotts UK, Bramford, Suffolk). Experiments were carried out in a glasshouse compartment set to 25/15 °C day/night. The plants were watered with plain tap water, with the only added nutrition being that added to the compost at manufacture. Lines that failed to germinate (14 lines) were re-sown. Forty days after sowing, one or two plants were harvested from each pot. Fresh weight of the above ground plant were recorded. Those plants that were re-sown were 47 days old, (21 September) and the parent lines reached 54 days old, (21 September) at harvest. Each harvested plant was placed in a coded bag and put in a drying oven for 72 hours at 80 °C. These samples were then weighed for dry weight, milled, sub-sampled and then subjected to a micro-Kjeldahal digest. Material from this digest was then analysed for macro and micronutrients using ICP-ES (Section 3). Carbon and total nitrogen concentration was measured using a combustion technique (see Section 3). Mean values of shoot fresh weight, shoot dry weight and shoot P concentration were made. QTL analyses were performed using mapping software (marker regression software, Kearsey & Hyne, 1994) and interval mapping by regression (Haley & Knott, 1992). Doubled haploid lines are an excellent resource on which to conduct QTL mapping since alleles are homozygous at all loci and, like RILs, they are genetically immortalised when propagated (Bettey et al., 2000).

4.3.1 Results & Discussion

There was two-fold variation in parents of DH parent lines in PUE (Fig. 31) and up to 15-fold variation in PUE amongst the 57 lines of the NB population harvested (Fig. 32). The proportion of variation in PUE that could be attributed to genetic factors was 33%. There are significant QTL for PUE in *B. oleracea* on linkage groups 6, 7 and 9 (Fig. 33). These preliminary data need to be supported in repeat experiments, so that the heritability of the trait, and the contribution of each QTL to the genetic component of the overall variance for each trait, can be determined with greater confidence. These data accord with studies recently published on other crops. For example, significant genetic variation in PUE and/or major genetic loci impacting on PUE have recently been identified in rice, wheat, sorghum and soybean (Horst et al., 2001).

Fig. 31

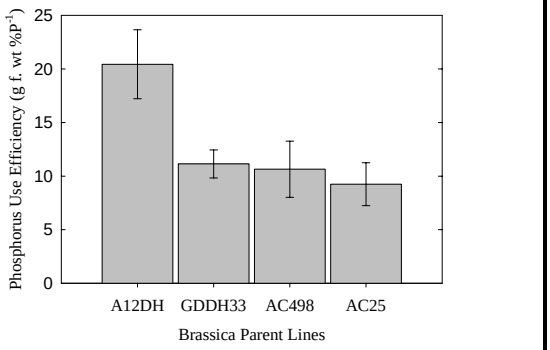


Fig. 31 P-use efficiency of *B. oleracea* doubled-haploid lines. DH lines were grown for 6 weeks in a sand / compost mixture (mean ± s.e.m, n = 6 plants).

Fig. 32

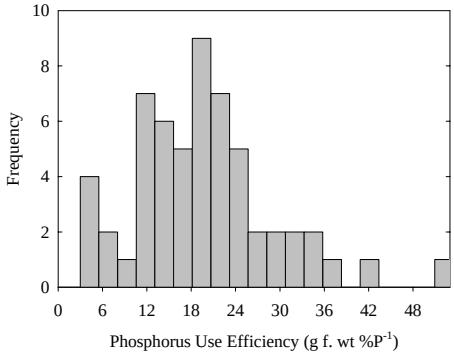


Fig. 32 Frequency distributions of P-use efficiency trait of 58 doubled-haploid lines (DH) from the *Brassica oleracea* 'NB' mapping population (obtained from a cross between var. *italica* x var. *botrytis*). Plants were grown for c. 7 weeks in compost.

Fig. 33

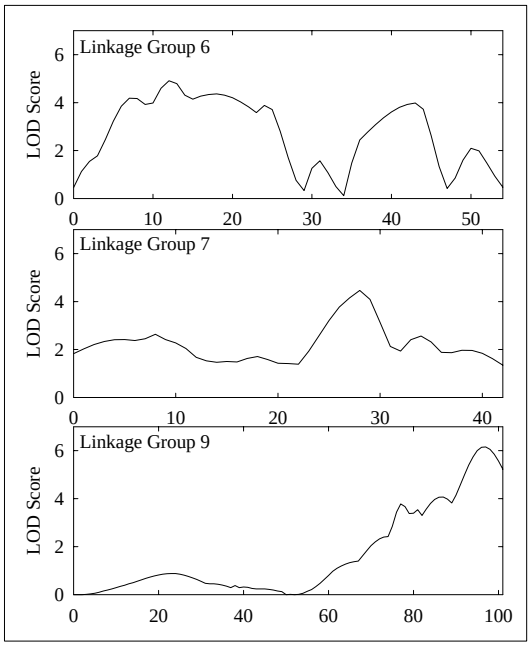


Fig. 33 QTL impacting on P-use efficiency on Linkage Groups 6, 7 and 9 of *Brassica oleracea*. Data were obtained from the 'NB' mapping population (obtained from a cross between var. *italica* x var. *botrytis*). Plants were grown for c. 7 weeks in compost. The QTL likelihood (variance ratio) was obtained using the Interval Mapping option of QTL café.

Future work will determine if putative chromosomal loci have a significant and reproducible effect. These will be confirmed and resolved using (i) alternative mapping populations (ii) *Brassica* substitution lines where these are available (iii) by determing homology and conservation of gene order with *Arabidopsis*. There are numerous possible opportunities for using this information to (1) match genotypes to their nutritional environment (e.g. identify P-efficient genotypes suited to low-input systems), or (2) provide information that can be used in crop improvement programmes.

4.4 Summary of Objective 5

- There is a significant genetic component to PUE in model Brassicaceae crops, as is the case for several staple food crops.
- Putative loci impacting on PUE under these conditions occur on Chromosomes I and IV in *Arabidopsis*, and on Linkage Groups 6, 7 and 9 in *Brassica oleracea.*
- The locus on Chromosome I in *Arabidopsis* coincides with observations from other studies.
- In *Arabidopsis*, we suggest that putative loci are tested and resolved in a homogeneous substrate (preferably agar).
- We recommend that loci identified in *Arabidopsis* are fine mapped using emerging technologies.
- Genetic loci can be used to develop molecular markers for improving PUE in breeding programmes, or, can be used to screen existing germplasm for P-efficient varieties.
- Although modern varieties of staple crops are generally more nutrient-efficient than older cultivars (reviewed by Baligar, 2001), advances in molecular genetics are opening up new approaches to increasing PUE.

4.5 Knowledge / technology tranfer from meeting this Objective

- Transferred skills and knowledge to train a BBSRC Student to map cation accumulation traits in *Arabidopsis* (2001-2004).
- Met with Henry Doubleday Reseach Association to discuss potential of selecting / breeding nutrient-efficient varities of vegetables for organic production systems (February 2002, Martin Broadley).
- Used preliminary results to support a more applied project application to DEFRA, to systematically study nutrient efficiency traits in vegetables.

APPENDIX I

The following papers and oral presentations have been produced during the lifetime of the project. Where work is directly related to Objectives in HH1408SFV, DEFRA support has been acknowledged in the paper. These papers are highlighted in **bold type**.

*Refereed papers published during project (*invited review):*

***White, P.J., Swarup, K., Escobar-Gutiérrez, A.J., Willey, N.J., Broadley, M.R. (2002). Selecting plants to minimise radiocaesium in the food chain. *Plant & Soil*, invited review.**

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White, P.J., Whiting, S.N., Baker, A.J.M., Broadley, M.R. (2002). Does zinc move apoplastically to the xylem in roots of *Thlaspi caerulescens*? *New Phytologist*, **153**, 201-207.

Broadley, M.R., Burns, A., Burns, I.G. (2002a). Relationships between P forms and plant growth. *Journal of Plant Nutrition*, 25, 1075-1088.

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***White, P.J., Broadley, M.R. (2000). Tansley Review 113: Mechanisms of caesium uptake by plants. *New Phytologist*, 147, 241-256.**

*Papers submitted during project (*invited review):*

Broadley, M.R., Burns, A., Escobar-Gutiérrez, A.J., Burns I.G., White P.J. Effects of nitrogen content on the growth of butterhead lettuce (*Lactuca sativa* L. cv. Kennedy). *Plant & Soil*, submitted.

Walker, R.L., Burns, I.G., Moorby, J., Mead, A. Dependence of plant growth rate on tissue concentration of nitrate and organic-N in lettuce grown under high and low light conditions. *Journal of Experimental Botany*, submitted.

*Whiting, S.N., Broadley, M.R., White, P.J. (2002). Applying a solute transfer model to phytoextraction: Zinc acquisition by *Thlaspi caerulescens*. *Plant & Soil*, invited review.

Oral presentations during project:

White P.J., Broadley M.R. (2001). Mechanisms of radiocaesium and radiostrontium uptake by plants. In: *NERC Co-ordination Group on Environmental Radioactivity, Open Annual Meeting, 12th-14th Sept., 2001, Imperial College of Science and Technology, London.*

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