Nitrogen Supply Influences Carbohydrate Partitioning of Pepper Seedlings and Transplant Development

Beny Aloni, Tamara Pashkar, and Lea Karni

Department of Vegetable Crops, Institute of Field and Garden Crops, Agricultural Research Organization, The Volcani Center, P.O.B. 6 Bet Dagan, Israel

Jaleh Daie¹

Department of Crop Science, Cook College, Rutgers University, New Brunswick, NJ 08903-0231

Additional index words. transplant recovery, sugars, assimilates, partitioning, hardening, *Capsicum annuum*

Abstract. We investigated the effects of N nutrition on growth and carbohydrate partitioning of pepper (*Capsicum annuum* L., cv. Maor) seedlings in the greenhouse and on their subsequent recovery and development after transplanting. Seedlings received 0, 30, 100, or 200 mg N/liter for 14 days, after which they were transplanted and received 100 mg N/liter. Nitrogen levels below 100 mg·liter⁻¹ inhibited shoot growth and leaf chlorophyll content; both were severely inhibited in the absence of supplemental N. Root growth had a negative relation with N supply; an enhanced root : shoot ratio was observed under low-N regimes. On a unit-leaf-area basis, CO₂ fixation was not affected when N was present; however, it was greatly inhibited in the absence of N. Changes in the leaf starch and soluble sugar concentrations occurred as a function of N supply and leaf age. In the roots, low N led to lower sucrose and higher levels of hexose and starch. More sucrose was transported and accumulated into leaf veins of low-N tissue. Exogenously supplied ¹⁴C-labeled sucrose was rapidly converted into starch in the low-N tissue. Seedlings that received 100 mg N/liter had the highest post-transplant growth rate and flowered earlier. Carbohydrate status of young pepper seedlings influenced their post-transplant recovery. Optimal N supply is essential for full recovery and development of transplants.

In many parts of the world, pepper seedlings grown in the greenhouse are established in the field by transplanting. Transplant shock occurs because field conditions are often more stressful than those in the greenhouse. Irrigation, fertilization, and other cultural practices during the initial growth of the seedlings in the greenhouse may influence the quality of the transplants and their rate of recovery after transplanting. To reduce the impact of transplant shock on the final fruit yield and harvest maturity, seedlings are hardened by nutritional deficiencies or drought stress, which inhibits excessive growth and increases seedling uniformity (Bar-Tal, 1987). While hardening treatments have been shown to enhance survival rate of transplanted seedlings (McKee, 1981), in many cases, they may negatively impact post-transplant recovery and crop development. Our objective was to determine the effect of several N levels on growth and assimilate-partitioning patterns of pepper seedlings in the greenhouse and to monitor their post-transplant recovery and development.

Materials and Methods

Plant material and growth conditions. Seeds of 'Maor' bell pepper, obtained at the Volcani Center, Bet Dagan, Israel, were germinated in Speedling trays (30-ml cell volume) filled with a 1 vermiculite : 1 peat mixture (v/v). Seedling growth and all other post-transplant experiments were carried out in a greenhouse with day and night averages of 25 and 18°C, respectively; daylength was 14 h and the natural light conditions provided $\approx 1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at midday. Seedlings were irrigated with

tap water until the second true-leaf stage, after which nutritional treatments were initiated. Each nutrient regime was applied to two Speedling trays (200 transplants) and continued for 14 days after which seedlings were transplanted into 3-liter pots. The design was a completely randomized arrangement. Twenty one-plant replicates of uniformly sized seedlings were transplanted from each nutritional treatment. The nutrient solution was full-strength Hoagland (Hoagland and Arnon, 1950) in which the KNO₃ level was modified to give a final N concentration of 0, 30, 100, or 200 mg·liter⁻¹. Potassium was supplied at a constant rate to all treatments with KH₂PO₄. The level of K in the full-strength Hoagland solution presumably was sufficient to mask the differences in K level in treatments that received low or no KNO₃. To have a continuous supply of nutrients and to avoid water deficit, trays were floated over 100-liter containers filled with the appropriate nutrient solution throughout the experiment. Shoot and root dry weights were determined after drying the tissue at 70°C for 48 h. Samples for biochemical determinations were taken at the same time and were quickly frozen in liquid N. After transplanting to larger pots, all seedlings were irrigated daily with full-strength Hoagland solution for 30 days. Shoot fresh weight, plant height, number of flowers, and the day of the first flower at anthesis were determined at the end of the 30-day recovery period.

Application of ¹⁴C-labeled CO₂. ¹⁴C-labeled CO₂ fixation, and ¹⁴C-labeled assimilate distribution. To measure the CO₂ fixation capacity of the seedlings, a fully expanded mature source leaf (the second true leaf from the base) was labeled with 5 ml of ¹⁴C-labeled CO₂. Labeled CO₂ was generated by mixing 50 μCi (1 Ci = 37 GBq) ¹⁴C-labeled NaHCO₃ with 4 ml concentrated HCl in an air-tight flask. The labeled CO₂ was withdrawn with a syringe and injected into a 30-ml plastic chamber that was tightly attached to the leaf's upper surface. The leaf was subjected to a 10-min pulse of ¹⁴C-labeled CO₂, after which it was detached to determine the total fixed label (measured by scintillation counting). During this 10 min, export of ¹⁴C out of

Received for publication 19 Feb. 1991. This is article no. D-15192-1-91 of the New Jersey Agricultural Experiment Station. Funding was provided by BARD (Binational Agricultural Research and Development) Grant no. I-1068-86 to B.A. and J.D. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹To whom reprint requests should be addressed.

the leaf was negligible. Therefore, the label measured in the leaf represented the total newly fixed carbon. To prepare tissue for scintillation counting, it was first digested and bleached in 4 ml of a 60 H₂O₂ : 40 HClO₄ solution (v/v) at 50°C until complete discoloration. After it cooled, 0.5 ml from each sample was mixed well with 4 ml of scintillation solution (Insta gel 11, Packard, Calif.) and radioactivity was counted in a scintillation counter (BETAMatic 1, Kontron Analytical, Switzerland).

Partitioning of labeled assimilates into various organs was determined by exposing the whole seedlings to labeled CO₂. Each seedling was enclosed for 1 h in a 2-liter glass chamber containing ¹⁴C-labeled CO₂. The seedlings were then divided into their various parts and processed for counting as described above.

Determination of sugars, starch, and chlorophyll. The leaves from each seedling were divided into four groups: A) leaves that existed before N regimes started and that were mature at the end of the experiment; B) leaves that continued to grow during the experiment and became fully expanded at the termination of the experiments; C) young expanding leaves that were 2 to 3 cm long at the end of the experiment (30% of fully expanded); and D) the youngest leaves, <1 cm long. At sampling, groups A and B were mature source leaves; group C, transitional leaves (they had CO₂ fixation ability but were still importing some assimilates) (Pitcher and Daie, 1991); and group D, sink leaves. To determine the carbohydrates, ≈200 mg frozen leaf or washed root tissue was extracted three times with 80% ethanol at 80°C. The combined extracts were evaporated to dryness and redissolved in 2 ml distilled water and 50- to 200-μl aliquots were used for sugar determination. Reducing sugars were determined calorimetrically using dinitrosalicylic acid (Miller, 1959). Sucrose was determined using the anthrone reagent method as modified for determination of nonreducing sugars (Van Handel, 1968). Starch was determined following digestion of the ethanol-insoluble residue with amyloglucosidase (Dinar et al., 1983). Chlorophyll was measured by homogenizing the tissue in chilled acetone followed by centrifugation. Absorbance was then measured at 652 nm (Arnon, 1949).

¹⁴Carbon-labeled sucrose uptake and partitioning in the leaf. Phloem loading was estimated by uptake studies. Sucrose uptake was measured in leaf disks (7 mm in diameter) prepared from fully expanded source leaves of seedlings using a sharp cork borer. The disks were gently stirred for 60 min in the base medium that contained 20 mM 2N-morpholinoethane sulfonic acid-Bis Tris-propane (MES-BTP) buffer, adjusted to pH 5.5, 2 mM KCl, 1.0 mM CaCl₂, and 200 mM mannitol as the osmoticum. Paramercuricbenzosulfonate (PCMBS) was used as an inhibitor of the sucrose carrier. After the 60-min incubation, disks were exposed to 1 mM PCMBS for 10 min and rinsed three times in 10 ml base medium. Five disks (in three replicates) were then placed in 3 ml of uptake solution, which was the base medium plus 2 or 20 mM sucrose and uniformly labeled ¹⁴C-labeled sucrose (specific activity, 5 Ci/mole). The final specific activity of sucrose in the uptake solution was 0.1 μCi/μmole. ¹⁴Carbon-labeled sucrose uptake was allowed for 1 h at 25°C, after which excess radioactivity from cut surfaces and free space was removed by washing the disks three times with 10 ml of the base solution (no sucrose) (Saftner et al., 1983). The disks were then digested for scintillation counting as described above.

To determine the partitioning of the ¹⁴C-labeled sucrose between the ethanol-soluble (sugars) and -insoluble (starch) frac-

tions, leaf disks from each group were incubated with 2 mM ¹⁴C-labeled sucrose, as described above, for 2 h. After the triple rinse/wash procedure to remove excess radioactivity, labeled sugars in the disks were extracted with 80% boiling ethanol (three times). The extracts were combined, evaporated to dryness, and dissolved in 0.5 ml distilled water before being mixed with 4 ml of scintillation cocktail. Labeled starch was determined from the solid tissue residue that was washed three times with distilled water then digested with amyloglucosidase (EC 3.2.1.3) (Dinar et al., 1983). After the enzymatic digestion, the soluble material was concentrated by rotoevaporation to a volume of 2 ml from which 0.5 ml was counted as described above.

Results

Seedling growth, ¹⁴C-labeled CO₂ fixation, and carbohydrates. Nitrogen concentration in the nutrient solution had significant effects on the growth of the seedlings before transplanting (Table 1). Leaf count and weight increased with increasing N, but root fresh and dry weights were lower at 200 than at 30 mg N/liter. Total dry matter in leaves progressively increased from 0.03 to 0.21 g as N increased from 0 to 200 mg·liter⁻¹. Roots grown without N were smaller than those grown with 30 or 100 mg·liter⁻¹. Because total root growth was inhibited at 200 mg N/liter, the root : leaf ratio decreased with increasing N (Table 1), indicating a substantial shift in overall assimilate allocation within the seedlings.

Differing N levels resulted in seedlings of different sizes and, consequently, in variations in CO₂ fixation on a per plant basis (Fig. 1). Due to a larger leaf area, on a per seedling basis, photosynthetic capacity increased as a function of N supply. The highest N level did not enhance total photosynthetic capacity relative to seedlings that received 100 mg N/liter. On a unit-surface-area basis, ¹⁴CO₂ fixation rates of the source leaves of seedlings that received N did not change, and low photosynthetic rates were observed only in the absence of N. Changes in photosynthetic capacity per unit leaf area showed a pattern similar to those of leaf chlorophyll content; only in the absence of any N did chlorophyll content decrease significantly (30%) compared to those that received 200 mg N/liter.

In the absence of N in the solution, starch and soluble sugars accumulated (Fig. 2). At 30 mg N/liter, starch and hexose levels were higher than those in seedlings treated with 100 or 200 mg N/liter. Starch and sugar contents were relatively stable at higher N levels. Overall, leaf starch content was more sensitive to N supply than were sugar levels.

To monitor carbohydrate partitioning within the leaves in more detail, starch and sugars were measured in leaves of various developmental stages (groups A, B, C, and D) from seedlings subjected to the 14-day N regime. In these experiments, only one level of N (100 mg·liter⁻¹) was compared to controls. In N-deficient seedlings, starch levels increased significantly as leaves aged (Fig. 3), suggesting that young leaves may not have the ability to synthesize and/or store starch. At 100 mg N/liter, starch levels remained low in leaves of all ages; their highest starch content was 10% of that in the oldest N-deficient leaves. Regardless of the leaf age, more sucrose than hexose accumulated in N-deficient leaves than in those receiving N (Fig. 4). Hexose levels remained relatively stable in all leaf types from the two N regimes, except they were highest in the youngest leaves of deficient seedlings. In the roots, hexose levels were 5 times and starch levels 2 times higher in plants subjected to low and deficient N regimes compared to those receiving higher N (Table 2). Sucrose concentration, however, increased nearly 7

Table 1. The effect of N concentrations in the nutrient solution on growth characteristics of pepper seedlings after 14 days of nutritional treatment. Values in parentheses are percent dry matter. Data points are means of at least 10 replicates $\pm$ SE.

N concn (mg·liter ⁻¹)	No. of leaves	Stem length (cm)	Root wt (g)		Total leaf wt (g)		Root : leaf dry wt
			Fresh	Dry	Fresh	Dry	
0	4.8 $\pm$ 0.2	5.5 $\pm$ 0.7	0.52 $\pm$ 0.08	0.045 (8.6) $\pm$ 0.008	0.22 $\pm$ 0.04	0.03 (13.6) $\pm$ 0.005	1.50
30	7.2 $\pm$ 1.0	8.5 $\pm$ 0.9	0.72 $\pm$ 0.05	0.073 (10.1) $\pm$ 0.012	0.71 $\pm$ 0.06	0.10 (14.0) $\pm$ 0.03	0.73
100	8.1 $\pm$ 1.0	12.1 $\pm$ 1.5	0.67 $\pm$ 0.03	0.061 (9.1) $\pm$ 0.008	1.42 $\pm$ 0.2	0.15 (10.6) $\pm$ 0.02	0.40
200	8.6 $\pm$ 0.8	14.3 $\pm$ 1.2	0.47 $\pm$ 0.06	0.038 (8.1) $\pm$ 0.006	1.76 $\pm$ 0.2	0.21 (11.9) $\pm$ 0.02	0.18

Fig. 1. Effect of N concentration in the nutrient solution on the chlorophyll content (●—●) and on ¹⁴C-labeled CO₂ fixation, calculated on a unit-leaf-area (●—●) or per plant (○—○) basis. Measurements were made on source leaves of pepper seedlings 14 days after the onset of the nutritional treatments. Data points are means of five replicates. Bars are SE.

fold in plants that received high N compared to those that received no N (Table 2).

¹⁴Carbon-labeled sucrose transport. The shift in carbohydrate metabolism, as well as in allocation patterns of assimilates between shoots and roots, may have involved changes in relative amounts of sucrose exported out of source leaves. This possibility was investigated by estimating phloem loading of ¹⁴C-labeled sucrose in leaf disks under conditions of low external sucrose concentrations (2 mM) in which sucrose uptake occurs predominantly into the phloem veins (Giaquinta, 1976, 1977). We first verified that sucrose uptake kinetics in pepper leaves were similar to other tested species (Daie and Wyse, 1985). Indeed, we observed the typical biphasic kinetics (Fig. 5) with a saturable component at sucrose concentrations of <10 mM and a linear component at higher sucrose concentrations. Furthermore, only the saturable and not the linear component was inhibited by 1 mM PCMBs. In the presence of 2 or 20 mM PCMBs (sucrose uptake is mainly by mesophyll cells), sucrose uptake rates were higher in low-N leaves (0 to 30 mg·liter⁻¹) than in leaves of plants that received 100 or 200 mg N/liter (Table 3).

Fig. 2. Effect of N concentration in the nutrient solution on the content of hexose (○), sucrose (●), and starch (△) in the source leaves of pepper seedlings 14 days after the onset of the nutritional treatments. Data points are means of five replicates. Bars are SE.

Fig. 3. Effect of 0 (●) or 100 (○) mg N/liter on starch content in leaves of four development stages. Interpretation of each letter in the abscissa is given in the Materials and Methods section. Data points are means of five replicates. Bars are SE.

During a 2-h incubation of leaf disks in ¹⁴C-labeled sucrose, as much as 10% of the labeled sucrose taken up by low-N leaves was converted to starch (Fig. 6). In plants that received 100 or 200 mg N/liter, conversion of labeled sucrose to starch was only

Fig. 4. Effect of 0 (▲, ●) and 100 (△, ○) mg N/liter on the concentrations of sucrose (---) and hexose (—) in leaves of four developmental stages of pepper seedlings 14 days after the onset of the nutrient treatments. Interpretation of the letters in the abscissa are given in the Materials and Methods section. Data points are means of five replicates. Bars are SE.

Table 2. Carbohydrate status of the root tissue of pepper seedlings exposed for 14 days to several N concentrations. Data points are means of at least 10 replicates $\pm$ SE.

N concn (mg·liter ⁻¹)	Hexose (mg·g ⁻¹ fresh wt)	Starch	Sucrose
0	0.5 $\pm$ 0.1	1.18 $\pm$ 0.20	3.1 $\pm$ 0.45
30	0.4 $\pm$ 0.2	1.10 $\pm$ 0.12	5.2 $\pm$ 0.40
100	0.1 $\pm$ 0.03	0.62 $\pm$ 0.07	21.3 $\pm$ 1.05
200	0.1 $\pm$ 0.05	0.50 $\pm$ 0.07	20.7 $\pm$ 2.30

Fig. 5. Concentration-dependent kinetics of sucrose uptake in leaf disks of pepper. Disks were treated for 10 min with (○) or without (●) 1 mM PCMBs and exposed to ¹⁴C-labeled sucrose for 1 h in a buffered (pH 5.5) uptake solution. Data points are means of three replicates, five disks per replicate.

2%. These results were consistent with the observed shift in carbohydrate metabolism favoring starch biosynthesis and accumulation in mature leaves of N-deficient plants. The data also suggested that the increase in the root : shoot ratio in plants

Table 3. ¹⁴Carbon-labeled sucrose uptake in pepper leaf disks obtained from source leaves of seedlings treated with nutrient solutions of various N levels. Labeled sucrose uptake was measured for 1 h in the presence of either 2 or 20 mM sucrose. Data points are means of three replicates $\pm$ SE.

N concn (mg·liter ⁻¹)	Sucrose (mM)	
	2	20
	Sucrose uptake (μmol/g fresh wt per h)	
0	3.4 $\pm$ 0.3	6.9 $\pm$ 0.9
30	2.9 $\pm$ 0.3	6.7 $\pm$ 0.7
100	2.4 $\pm$ 0.2	5.1 $\pm$ 0.4
200	1.8 $\pm$ 0.2	4.8 $\pm$ 0.2

Fig. 6. Effect of N concentration on the partitioning of ¹⁴C-labeled sucrose into ethanol-insoluble (starch) fraction in leaf disks prepared from source leaves of pepper seedlings. Labeled sucrose (2 mM) was introduced to leaf disks for 2 h, after which partitioning into the ethanol-insoluble fraction was determined. Data points are means of three replicates. Vertical bars are SE.

Table 4. Effect of pretransplant N treatments on the post-transplant development of pepper seedlings. Measurements were made 30 days after transplanting seedlings into 3-liter pots. Data points are means of 20 replicates $\pm$ SE.

N concn (mg·liter ⁻¹)	Shoot fresh wt (g)	Plant ht (cm)	Time to first flower in anthesis (days)
0	15.0 $\pm$ 3.1	23 $\pm$ 3	No flowers
30	25.3 $\pm$ 4.0	47 $\pm$ 8	26
100	48.7 $\pm$ 5.4	58 $\pm$ 3	18
200	54.0 $\pm$ 9.1	61 $\pm$ 7	21

treated with low or no N (Table 1) may have resulted from higher export rates out of those leaves to the roots.

Seedling development after transplanting. Maximal vegetative growth of seedlings 1 month after transplanting was at the highest N level (Table 4). The development of N-deficient seedlings was irreversibly impaired since their apical meristem deteriorated and did not produce new leaves or flowers. Although development of seedlings receiving low pretransplant N (30

mg·liter⁻¹) was slower than those grown with 100 or 200 mg, they were able to grow and produce new leaves and flowers. Under the conditions of this experiment, 100 mg N/liter appeared to be optimal since these transplants were the first to flower and reach anthesis. Flowering in these seedlings was 2 to 3 days earlier than in those grown with 200 mg N/liter and 4 to 5 days earlier than those grown with 30 mg.

Discussion

Nitrogen deficiency is known to induce two distinct changes in carbon partitioning in leaves: a) starch accumulation (Ariovich and Cresswell, 1983; Rufty et al., 1988) and b) enhanced translocation and export of assimilates out of leaves to roots, resulting in increased root : shoot ratio (Browwer, 1962; Ingestad, 1979). In the present study, large quantities of starch and sugars accumulated in leaves of plants that did not receive N.

Sucrose uptake by leaf disks of low-N seedlings was higher than that from high-N seedlings, suggesting that the sucrose carrier was able to transport sucrose more efficiently in those seedlings, hence phloem loading was enhanced. We suggest that membrane transport of sucrose per se and loading into the phloem veins did not appear to be a limiting factor for sucrose export and translocation in N-deficient seedlings. This observation agrees with those in reports that indicate that sucrose loading is enhanced under other stressful conditions, such as low turgor (Daie and Wyse, 1985; Smith and Milburn, 1980).

The shift in leaf carbohydrate metabolism from sucrose to starch synthesis would be expected to decrease the availability of C for export to all sink organs, including young leaves and roots. However, export of C out of N-stressed leaves to the roots appeared to be enhanced. Also, under low-N conditions, C was readily compartmentalized as starch in the chloroplast, so that some of the newly formed carbohydrates were not available for export to support shoot growth. Together, these data indicated that under N stress, roots are more competitive sinks for assimilates than are young leaves and the meristem. Sucrose content in the roots of N-deficient seedlings was much lower than that in the roots of high-N seedlings. Further, at low N levels, no substantial starch accumulated in the roots. Sucrose exported to the roots apparently was rapidly hydrolyzed to support growth, presumably due to enhanced invertase activity.

Differences in initial growth and assimilate partitioning of seedlings may be significant to their post-transplant recovery and development. At the time of transplanting, there is high sink demand for a continuous supply of assimilates. Such assimilates may come from newly fixed CO₂ or from storage pools; both sources may become limited under low-N conditions. Therefore, post-transplant recovery of N-deficient seedlings would be expected to be slow. Our data indicate that N-deficient seedlings were indeed slower to recover even when sufficient N was supplied after transplanting. Application of optimal N concentrations to pepper seedlings before transplanting may ensure their rapid reestablishment, recovery, and development in the field.

Nitrogen deficiency caused major alterations in assimilate-partitioning patterns of pepper seedlings. However, these effects may have been through alterations in other key processes. For example, N-deficient plants were shown to have reduced con-

centrations of cytokinins in the roots (Horgan and Wareing, 1980; Salama and Wareing, 1979; Wagener and Michael, 1971). It has also been shown that leaves of N-deficient plants have high abscisic acid concentrations (Daie et al., 1979; Radin et al., 1982). These hormonal changes may be part of a mechanism that controls assimilate partitioning, as we observed.

Literature Cited

- Ariovich, D. and C.T. Cresswell. 1983. The effect of nitrogen and phosphorus on starch accumulation and net photosynthesis in two variants of *Panicum maximum* Jacq. Plant Cell Environ. 6:657-664.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.
- Bar-Tal, A. 1987. Effect of pepper (*Capsicum annuum*) seedling nutrition in the nursery on transplants development, establishment in the field and fruit yield. PhD Diss., Hebrew Univ. of Jerusalem.
- Browwer, R. 1962. Nutritive influences on the distribution of dry matter in the plant. Neth. J. Agr. Sci. 10:399-408.
- Daie, J., S.D. Seeley, and W.F. Campbell. 1979. Nitrogen deficiency influence on abscisic acid in tomato. HortScience 14:261-262.
- Daie, J. and R.E. Wyse. 1985. Evidence on the mechanism of enhanced sucrose uptake at low cell turgor in leaf discs of *Phaseolus coccineus*. Physiol. Plant. 64:547-552.
- Dinar, M., J. Rudich, and E. Zamski. 1983. Effects of heat stress on carbon transport from tomato leaves. Ann. Bot. 51:97-103.
- Giaquinta, R.T. 1976. Evidence for phloem loading from apoplast: Chemical modification of membrane sulfhydryl group. Planta Physiol. 57:872-875.
- Giaquinta, R.T. 1977. Phloem loading of sucrose: pH dependence and selectivity. Plant Physiol. 59:750-755.
- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method of growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.
- Horgan, J.M. and P.F. Wareing. 1980. Cytokinins and the growth response of seedlings of *Betula pendula* Roth and *Accr pseudoplatanus* L. to nitrogen and phosphorus deficiency. J. Expt. Bot. 31:525-532.
- Ingestad, T. 1979. Nitrogen stress in birch seedlings. Physiol. Plant. 45:149-157.
- McKee, J.M.T. 1981. Physiological aspects of transplanting vegetables and other crops. Hort. Abstr. 51:265-368.
- Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-428.
- Pitcher, L.H. and J. Daie. 1991. Growth and sink to source transition in developing leaves of sugarbeet. Plant & Cell Physiol. 32:335-342.
- Radin, J.W., L.L. Parker, and G. Guinn. 1982. Water relation of cotton plants under nitrogen deficiency. V. Environmental control of abscisic acid accumulation and stomatal sensitivity to abscisic acid. Plant Physiol. 70:1066-1070.
- Rufty, T.W., S.C. Huber, and R.J. Volk. 1988. Alterations in leaf carbohydrate metabolism in response to nitrogen stress. Plant Physiol. 88:725-730.
- Saftner, R.A., J. Daie, and R.E. Wyse. 1983. Sucrose uptake and compartmentalization in storage root of sugarbeet. Plant Physiol. 72:1-6.
- Salama, A.M.S. and P.F. Wareing. 1979. Effect of mineral nutrition on endogenous cytokinins in plants of sunflower. J. Expt. Bot. 30:971-981.
- Smith, J.A.C. and J.A. Milburn. 1980. Phloem turgor and the regulation of sucrose loading in *Ricinus communis* L. Planta 148:42-48.
- Van Handel, E. 1968. Direct microdetermination of sucrose. Anal. Biochem. 22:280-283.
- Wagner, H. and G. Michael. 1971. The influence of varied nitrogen supply on the production of cytokinins in the roots of sunflower plants. Biochem. Physiol. Pflanzen. 162:147-158.